Characterizing Gas Exchange and Assessing Feasibility of a New Lung Assist Device for Pre-Term and Term Neonates with Respiratory Distress Failure

By

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CHARACTERIZING GAS EXCHANGE AND ASSESSING FEASIBILITY OF A NEW LUNG ASSIST DEVICE FOR PRE-TERM AND TERM NEONATES WITH RESPIRATORY DISTRESS FAILURE

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ABSTRACT

Respiratory distress syndrome is a major cause of mortality among pre-term and term neonatal population. To overcome the limitations of current therapies, a new form of respiratory support termed the, "Artificial Placenta" has been proposed. The Artificial Placenta is a type of oxygenator that is attached postnatally via the umbilical vessels to provide pumpless respiratory support to pre-term and term neonates. To develop this concept, our group previously reported on a novel polycarbonate membrane lung assist device (LAD). To build upon its development, the objectives of this thesis are to determine the optimal interface for gas exchange, and characterize the gas exchange properties of the LAD under ambient and oxygen rich atmosphere. Subsequently, its feasibility was determined by studying the effects of extracorporeal flow rates on cardiovascular parameters and gas exchange performance was assessed in a newborn piglet model.

In vitro testing demonstrated that PDMS based membrane is the optimal interface for gas exchange in the LAD. In vitro testing of the LAD demonstrated 2.4 μL/min/cm² - 3.8 μL/min/cm² and 6.4 μL/min/cm²- 10.1 μL/min/cm² of O_2 and CO_2 transfer respectively under ambient air and oxygen rich atmospheric conditions. Based on these results, the LAD theoretically could provide 6-11% of metabolic O_2 while eliminating 18-26% of CO_2 in a newborn healthy pre term infant. Experiments in newborn piglet models achieved pumpless configuration with flow rates up to 60.9ml/kg/min without presenting

decompensation. Preliminary, in vivo gas exchange experiments demonstrated O_2 transfer of 3ul/min/cm^2, which matches closely to in vitro data.

A novel pumpless LAD is reported, which provides sufficient respiratory support. High extracorporeal flow rates with stable cardiovascular parameters demonstrate feasibility of the artificial placenta concept. This novel LAD could potentially serve as a rescue device when all other therapies such as nasal continuous positive airway and mechanical ventilation fail.

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CHAPTER 1

INTRODUCTION AND THESIS ORGANIZATION

1.1 Introduction

Respiratory distress syndrome, sometimes referred to as hyaline membrane disease, is a respiratory disorder of neonates. In healthy neonates, spontaneous breathing consists of two phases: inspiration and expiration. During inspiration alveolar pressure is less than atmospheric due to changes in the intrapleural pressure generated by the respiratory muscles and vice versa for expiration as shown in Fig 1.1 (Pearson Education Inc, 2011).

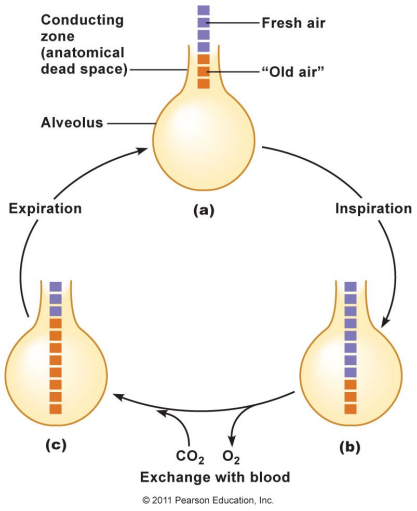


Figure 1.1 Diagram of gas exchange across the respiratory cycle (Pearson Education Inc, 2011)

Alveoli are the functional unit of gas exchange in the lung. They are covered with surfactants that prevent the lungs from collapsing upon expiration (Davidson et al, 2012:

Chapter 34). Respiratory distress syndrome is associated with surfactant deficiency, and complications can include hypoxia, respiratory acidosis, and metabolic acidosis (Davidson et al, 2012: Chapter 34). It is the most common form of respiratory disorder in premature infants typically below 35 weeks of gestational age (Rodriquez et al, 2002). Besides administration of antenatal steroids for progression of lung maturation, neonatal respiratory support is provided through different modalities such as: surfactant replacement therapy (SRT), mechanical ventilation, and nasal continuous positive airway pressure (nCPAP) (Rodriguez et al, 2002).

1.1.1 Surfactant Replacement Therapy

SRT is administered via an endotracheal tube, and results in rapid improvement in oxygenation (Davidson et al, 2012: Chapter 34). Important functions of surfactants in the normal lung include reducing tension in the alveolus, facilitating lung expansion, stabilizing the respiratory tract, defense against pathogens, and preventing the formation of edema (Donn and Sinha (2012), Chapter 26).

In its initial introduction, a trial conducted by Fujiwara et al, (1980) on n=10 mechanically ventilated pre term infants treated with SRT, demonstrated improved oxygenation and decreased requirement in ventilator support. Dunn et al, (1990) reported similar results in a large pre term infant population (n=75). Speer et al, (1992) further emphasized this, showing that multiple doses of surfactant vs. a single dose was effective

in improving oxygenation. He further went to report lower incidence of pneumothorax and mortality, however there was no reduction in rates of bronchpulmonary dysplasia and intracranial hemorrhage (Speer et al, 1992). Though there appears to be no increased risk of major disability (i.e. cerebral palsy) (Palta et al, 2000), there isn't a reduction in risk of other respiratory diseases later in adolescent life such as wheezing, asthma, and respiratory infection in early childhood (Engle, 2008). In addition rates of sepsis, and patent ductus arteriosus remain unaltered (Rodriguez, 2003).

1.1.2 Mechanical Ventilation

In 1963, the first newborn treated with mechanical ventilation survived without air leakage or cerebral abnormality (Bougatef, 2005). Mechanical ventilation provides respiratory support by administering the flow of gases via one of two connections: either by creating an artificial airway through the trachea by making an incision over the second tracheal ring, or through laryngoscopy and endotracheal intubation (Donn and Sinha, 2012: Chapter 14&16).

Figure 1.2 Mechanical ventilation intubation of infant (Sawin, n.d)

There is a potential 'pain' association related to mechanical ventilation resulting in unsynchronized breathing, suboptimal ventilation, and clinical instability (Barker 1996; Anand 1998). As well, implementation of this therapy requires a large amount of money, time and infrastructure, which is not always feasible in a developing country. This was clearly demonstrated by Riyas et al, (2003) who showed an overall survival rate of 51% in his observational study conducted in India, which is relatively lower than the 80-90% survival rate reported in the USA for infants >1000grams (Riyas et al, 2003).

Mechanical ventilation is associated with airway intubation complications, entry of pathogens into trachea, increased risk of pneumonia, minor lesions of the vocal chord, volutrauma (Slutsky, 1999), and bronchopulmonary dysplasia in infants <1250g (Linda et

al, 2000). This can lead to reduced lung compliance, altered surfactant structure/ function, and extra pulmonary air leak syndrome (Clark et al, 2001; Miller and Carlo, 2008).

Figure 1.3 Continuous positive airway pressure administration via binasal prongs (Intersurgical ltd, 2011)

1.1.3 Continuous Positive Airway Pressure

CPAP functions by applying positive pressure to the airways of a spontaneously breathing baby throughout its respiratory cycle. This helps maintain the spherical shape of the alveoli, resulting in progressive alveolar recruitment, stability, and reduction of intrapulmonary shunt (Bougatef, 2005).

In 1970, Gregory et al reported the use of endotracheal-tube CPAP in the treatment of respiratory distress in the newborn. In 1993, Agostino successfully treated a newborn with nasal modified CPAP (Bougatef, 2005). Verder et al, (1994) showed that CPAP along with SRT reduced the need for mechanical ventilation. In a single center study by Avery et al (1987), where CPAP was administered immediately after birth when

showing signs of respiratory distress syndrome they reported reduced risk of chronic lung disease for premature infants. CPAP also improves lung compliance, decreases airway resistance, improves alveoli curvature and conserves surfactants (Donn and Sinha, 2012: Chapter 26).

Historically CPAP was administered by different methods including: endotracheal intubation, face mask, head box while neck seal and nasal masks. Intubation was associated with high resistance, while obtaining a good seal around the neck or head was difficult for facemask and head box with neck seal method (Donn and Sinha, 2012: Chapter 26). Facemasks gave limited access to face and nasal masks were prone to damage the nasal bridge. Currently, the most common form of administration is to use short binasal prongs (Donn and Sinha, 2012: Chapter 26). The biggest disadvantage is CPAP must be individualized and altered to suit the baby's need, and cannot be used if the baby is apneic, bradycardiac, demonstrating respiratory failure, upper airway abnormalities or severe cardiovascular instability (Donn and Sinha, 2012: Chapter 26). Other complications include obstruction of the nose, over distension of the lung, nasal irritation, septum damage, mucosal damage, sepsis, skin irritation and fall out of CPAP nasal prongs (Donn and Sinha, 2012: Chapter 26).

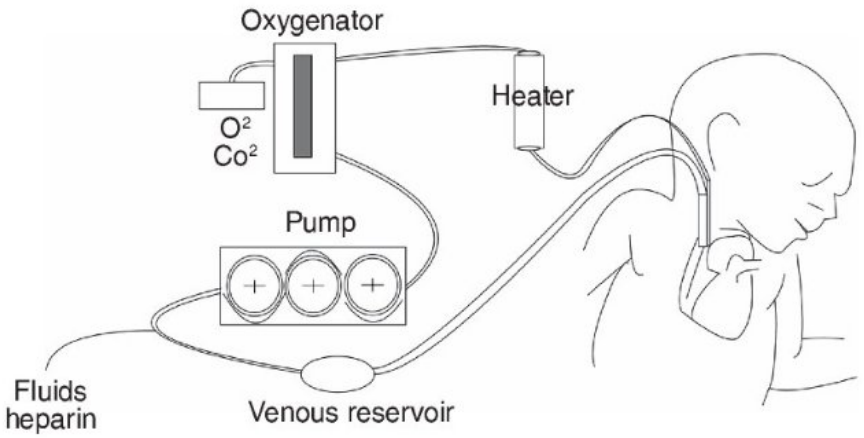


Figure 1.4 Extracorporeal membrane oxygenation administration (Gomez-Carao et al, 2010)

1.1.4 Extracorporeal Membrane Oxygenation

A final modality of respiratory support is extracorporeal membrane oxygenation (ECMO), which involves the creation of an extracorporeal bypass where the blood is oxygenated outside the body thereby bypassing the pulmonary system (Fig 1.4). In 1976, Barlett et al, reported the first mature newborn to be successfully treated with ECMO (Bartlett et al, 1976). The right jugular and carotid artery are used to create bypass flow of 80% of cardiac output therefore making it an invasive and technically complicated procedure. It is important to achieve adequate flow (100-120mL/kg/min) via mechanical pump. Also, ECMO is associated with a 3 fold increase in cost when compared to conventional treatment (Petrou et al, 2006). Its biggest disadvantage is its restriction of use to the term infant population with birth weights ≥ 2000g (Chapman et al, 2009), as pre term infants are not suitable due to the size of cannula required and due to increased risk of intracranial hemorrhage associated with the use of heparin (Cilley et al, 1986).

Despite the availability of all these modalities, each is associated with its own limitation with respect to the neonatal population. To overcome the limitations of these modalities, an alternative off shoot concept has arisen from ECMO, is the “Artificial Placenta”. This is a unique form of oxygenator application that seeks to provide a pumpless form of respiratory support to neonates via the umbilical vessels. It would be non invasive, characterized by high gas transfer, low flow resistance, and low priming volume. Potentially this form of technology could serve as a rescue therapy when other therapies such as nasal continuous positive airway pressure and mechanical ventilation fail.

1.2 Sequence of Chapters

The organization of the thesis is as follows:

Chapter 2 provides an overview of oxygenator history, design and development. A concise discussion regarding the advantages and disadvantages of different oxygenator types is also introduced.

Chapter 3 presents the proposed concept of "Artificial Placenta". A brief overview of the theory, and history is described. The main goals of the 'Artificial Placenta' are outlined.

Chapter 4 presents the proposed lung assist device design (oxygenator) based on theoretical parameters. A discussion of materials is also presented.

Chapter 5 clearly outlines the objectives of this thesis report.

Chapter 6 describes the fabrication technique used to assemble the final lung assist device.

Chapter 7 describes the experimental setup of the in vitro gas exchange testing of the lung assist device components, under ambient and oxygen rich atmosphere. Results are reported followed by discussion.

Chapter 8 describes the experimental setup for assessing the feasibility of the lung assist device in a newborn piglet model. Results are reported followed by a discussion.

Chapter 9 concludes the thesis by emphasizing the contribution of this research. A number of suggestions for future development of the device are proposed.

CHAPTER 2

OXYGENATORS: HISTORY, DESIGN AND DEVELOPMENT

The primary function of all oxygenators is to improve the oxygen saturation of the blood. Historically there have been two categories of oxygenators: direct and indirect oxygenators.

2.1 Direct Contact Oxygenators

In this form of oxygenator the flowing blood is in direct contact with ambient air allowing for direct diffusion of gases into the blood (Fig 2.1).

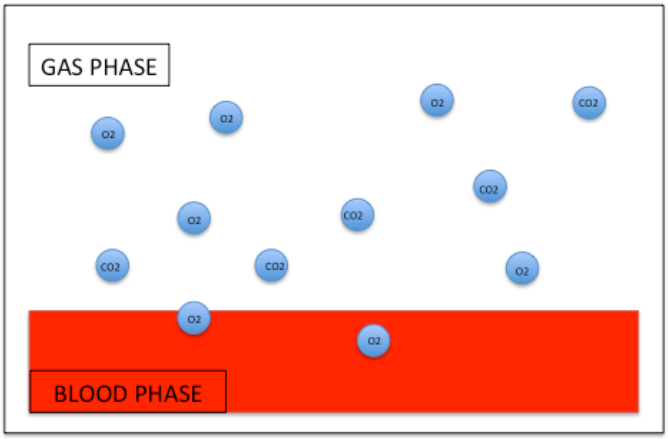


Figure 2.1 Diagram of gas exchange in direct contact oxygenator

Ludwig and Schmidt first introduced these types of oxygenators in 1868 (Lim, 2006) where they shook de-fibrinated blood with air in a balloon. Historically, there have been two forms of direct contact oxygenators: Bubble and Film.

2.1.1 Bubble

In 1882, Von Schroder created a system in which a venous reservoir collected blood, upon which air was introduced into the blood (Wylie, 1972). The oxygenated blood was then returned into an arterial chamber (Wylie, 1972). The first popular and clinically important type of bubble oxygenator was the, "De Wall Bubble Oxygenator" (DeWall, 2003).

The setup (Fig 2.2) consisted of a vertical 2ft long blood/oxygen tube, which had rubber cork perforated by 18 and 22 hypodermic needles inserted at the base of the vertical tube to produce bubbles (DeWall, 2003). Followed by a long diagonal large silicone coated de-bubbling chamber, a helical reservoir and a blood reservoir with its own pump to return blood to the patient (DeWall, 2003). An optimum bubble size of 2-7 mm was required for effective gas exchange (Nunn, 1993).

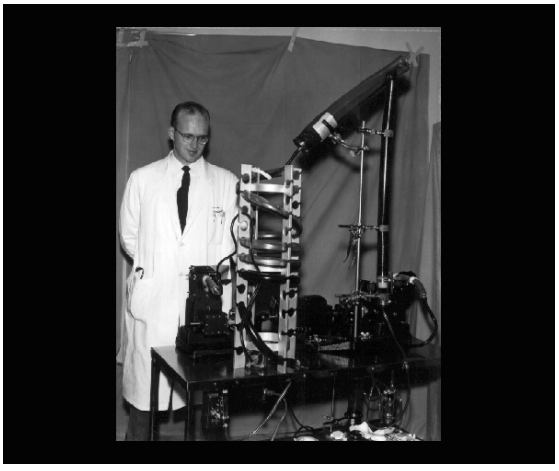


Figure 2.2 DeWall Bubble Oxygenator (DeWall, 2002)

The first clinical use of the bubble oxygenator was in May 1955 to treat a 3-year old boy with a ventricular septal defect (DeWall, 2002). It gained much popularity and in 1976 it was used in 90% of open-heart surgeries around the world (Lillehei, 1993). Its main advantage was that it provided a large surface area for gas exchange, and followed a simple design, it was inexpensive, had fairly low priming volumes which could be met with saline infusion and were disposable after one use (DeWall, 2002).

Flow rate determined the number of bubbles, which in turn influenced O_2 and CO_2 solubility. Small bubbles favoured oxygen transfer due to large surface to volume ratio however it did not eliminate carbon dioxide as well, whose diffusion dependent on the partial pressure difference between the blood and outside atmosphere (Lim, 2006). Though small bubbles appeared advantageous, they also had a tendency to remain in suspension thereby increasing the risk for air embolism (Lim, 2006).

As well reports of high blood trauma, including, protein denaturation made the technology quite risky (Wegner, 1997). Silicone coating of the de-foaming chamber allowed for break down of bubbles, but yet again introduced another foreign surface that contacted the blood (Wegner, 1997). Another limitation to this technology was that it could only be used up to 4 hours without complications (Wylie et al, 1972).

2.2.2 Film

The most popular of these was the Mayo-Gibbon pump oxygenator. The setup included cannulas inserted into the superior and inferior venae cava, which led into a venous reservoir, and blood was drawn through a manual vacuum system (Kirklin et al, 1956). Subsequently the blood was pumped up through narrow slits measuring 0.0008inch wide and 0.625inch high to the top of the oxygenator, and was released down a series of stainless steel screens (12 inches wide and 24 inches high), forming a thin film that was exposed to a flow of oxygen (Fig 2.3). The blood was filtered and returned back through a cannula into the patient's aorta (Kirklin et al, 1956).

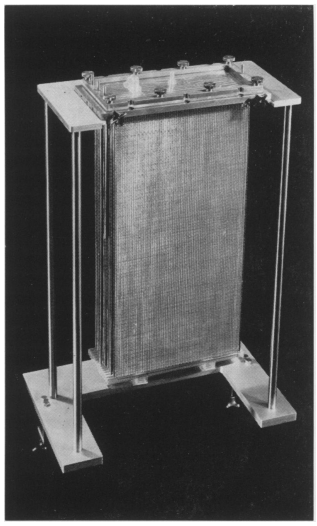


Figure 2.3 Mayo-Gibbon Pump Oxygenator (Kirklin et al, 1956)

The priming volume ranged from 2670mL for four screens- 3575mL with 14 screens (Jones, 1959). Kirklin subsequently went on to test its applicability in 40 patients undergoing intracardiac operations. Blood returned was 100% oxygen saturated with

patients returning to normal heart and lung function (Kirklin et al, 1956). Between March 1955-1959, 500 patients were treated with this technology and no patient was lost due to the system or its equipment (Jones, 1959), however disadvantages of this machine included: bulkiness, difficulty in sterilisation and operation, prone was to blood streaming, thereby diminishing the surface of the blood gas exchange and the requirement of relatively large priming volume (Lim, 2006).

Another type of film oxygenator was the Kay Cross rotating disc film oxygenator (Fig 2.4). It consisted of a series of 59 plastic coated stainless steel discs 0.15 inch thick, 4 13/16 inch in diameter, mounted 0.18 inch apart by means of stainless steel spaces on a central shaft (Cross et al, 1956). This setup used a series of discs across which a blood film was created. This was subsequently exposed to oxygen and the surface layer oxygenated and mixed with another layer of non-oxygenated blood on the next disc (Bjork et al, 1985).

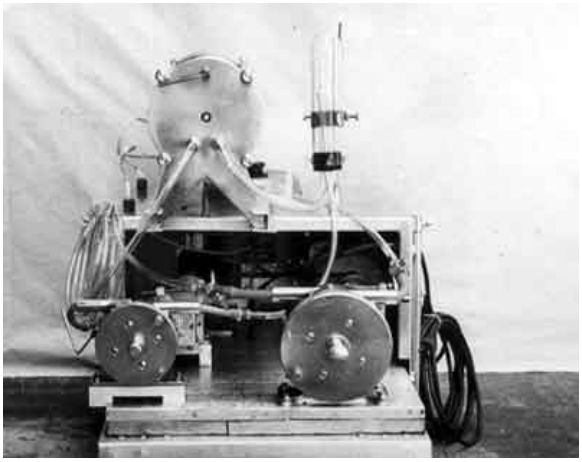


Figure 2.4 Kay Cross Oxygenator (Gomes et al, 2005)

A priming volume of 1400mL of was needed to immerse the discs, relatively lower than the Mayo Gibbon Pump oxygenator. The Total exposed surface area with 59 discs was 0.84m^2. Oxygen was supplied at 5L/min through perforated stainless steel tube mounted with in the cylinder above discs. Discs revolved at 120 rev/min and 110 square m^2 of filmed blood is exposed to oxygen per minute (Cross et al, 1956) .To approximate clinical relevance, 120 discs could oxygenate 5L/ min of blood (Bjork, 1985). This device became a popular choice among clinicians and was preferred over the disposable bubble oxygenator in part due to the perception that it caused less damage (Lim, 2006).

Though the surface area was large, the total blood volume in contact with gas was small relative to amount of blood in the device; in addition blood streaming limited the amount of contact (Iwahashi, 2004). Blood trauma, though lower than the bubble devices, required large surface area and high priming volume (Iwahashi, 2004).

2.2 Indirect Oxygenators

Gaylor (1988) defined membrane oxygenators as those devices in which, “gas exchange occurs across a hydrophobic, gas permeable membrane which separates the blood and ventilating gas phases” (Fig 2.5). The main motivation behind the development of these oxygenators was to reduce blood trauma, through introduction of an intermediary interface between permeating gas and blood phase. Though this introduces a longer diffusion distance to permeating gases, which necessitates a large gas exchange surface

area, risk of air embolism is also eliminated (Iwahashi, 2004). There are 3 forms of this technology: (a) spiral, (b) hollow fibre and (c) plate.

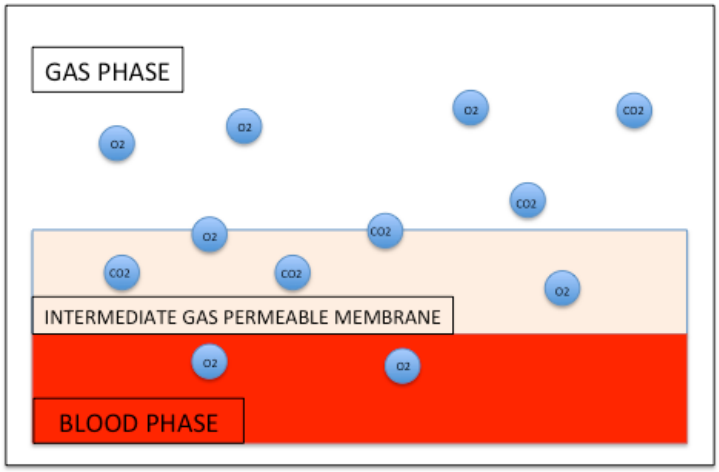


Figure 2.5 Design of indirect membrane oxygenator

2.2.1 Spiral Membrane Oxygenators

Initially developed by Kolff in 1956, blood flowed in a spiral pattern while oxygen flowed parallel to the axis of the cylinder. Kolobow further developed the design, by using a silicone rubber envelope to re- enforce with nylon knit wrapped around a central core, with an inlet/outlet for gas flow and sealed within an acrylic jacket (Edmund, 2002). Due to the high flow resistance the membrane deformed into the screen creating boundary irregularities and secondary flow effects, and improving gas exchange capability (Iwahashi 2004; Gaylor 1988). The priming volume was 100mL/m^2 and average perfusion rates of 6.4 days reported in lamb model (Zapol et al, 1971). In a study of 50 infants and children, this membrane type was reliable however platelet counts and fibrinogen concentrations decreased by 50 and 31% respectively (Saxena et al, 1977).

Though commercially available, they were quickly overcome by the development of hollow fibre technology.

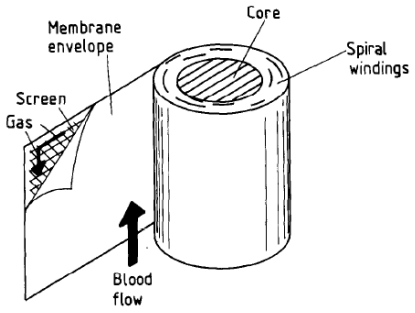


Figure 2.6 Kolobow Spiral Oxygenator (Gaylor, 1988)

2.2.2 Hollow Fibre

Developed in 1972, this technology consisted of polymeric hollow fibres bundled together and wrapped around a shell casing. The fibres ranged from 200-250um in diameter and 10-15cm long, with membrane thickness of 20-50 um (Wegner, 1997).

There are two types of hollow fibre layout: shell/tube configuration or cross flow hollow fibre (Fig 2.7) (Gaylor, 1988). In the shell configuration blood flows within the lumen of the bundle and gas flows over the fibre exterior counter current to the blood. In the cross flow design mixing of blood and enhancing of gas transfer is achieved by flowing the blood over the exterior surfaces of the fibre while gas is circulated within the lumen. For similar O_2 transfer rates, the membrane area required is 2-2.5 times smaller

than that for a shell and tube design (Gaylor, 1988) and associated with decreased blood trauma (Hollman et al, 1994).

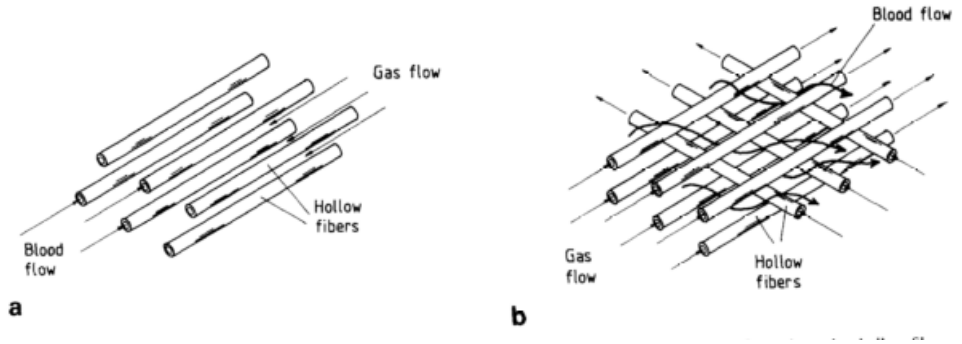


Figure 2.7 (a) Shell configuration and (b) cross flow design of hollow fibre technology (Gaylor, 1988)

In a study comparing hollow fibre (surface area 1.4m^2) to spiral coil membrane oxygenators (surface area 23.15m^2), the hollow fibre had not only a lower priming volume but also outperformed the spiral oxygenator along with respect to oxygen transfer (Rawitscher et al, 1973). Initial developments were hampered with plasma leakage through hydrophilic membrane. Pursued interest in finding suitable hydrophobic membranes that also did not limit gas transfer led to the discovery of silicone coated micro porous (0.1-0.2um) polypropylene, or polyethylene fibres (Iwahashi, 2004; Shimono et al, 1997; Gaylor, 1988).

The advantage of hollow fibre technology was that it induced passive mixing of blood, thereby improving gas exchange, and reducing the risk of particulate/air embolism (Bartlett, 2005). However leakage is a large problem to date, but non porous materials such as PMP (poly-4-methyl-1pentene) have been proposed as having equal gas exchange without the risk of leakage (Lim, 2006). Despite its wide used in the clinical setting this hollow fibre system is an invasive procedure requiring large volumes, mechanical pumps and systemic heparinization. Though it provides strong support during shock, sepsis and low blood volume (Bartlett, 2005), it can only be used on infants that are greater than 24 weeks and weight more than 2kg, thereby excluding use in the pre-term population (Lequier, 2004). In addition it cannot be used on patients who have demonstrated major congenital heart diseases and have reduced performance over time (Wegner, 1997).

2.2.3 Flat Sheet design

An example of this form is the Shiley M-2000 oxygenator (Fig 2.8). It consists of non woven polypropylene screen spacers between 60Z having a total surface area of 2.3m^2 and priming volume of 600mL (Bjork et al, 1985). Screen-induced mixing of the blood phase occurred to enhance the gas transfer (Gaylor, 1988) but again the device required access to the central vessels, and had a large priming volume not feasible for the neonatal population.

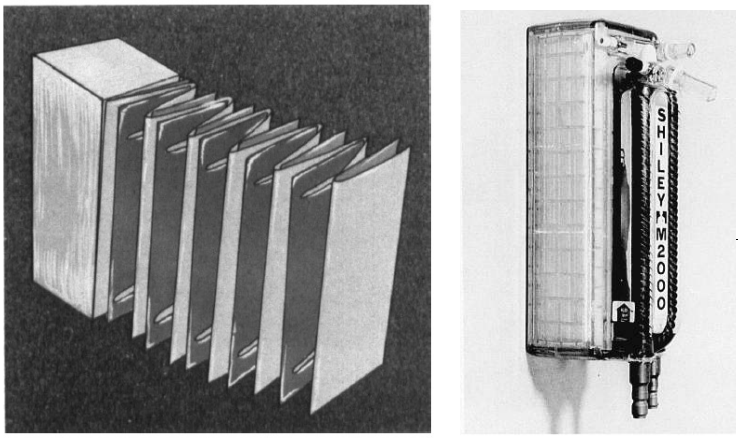


Figure 2.8 Shiley M-2000 Oxygenator (Cooper and Levin, 1987)

2.2.3.1 Microfluidic Approach

More recently, miniature or 'microfluidic' oxygenators of flat sheet design have been developed for cell culture applications to maintain dissolved oxygen concentration in culture media (Lam et al, 2009). The field of 'Microfluidics' involves the manipulation of liquids and gases in channels with dimensions of 10-100um, to create miniaturized systems for a variety of chemical, biological and medical applications (J. C McDonald et al, 2000). These include: DNA analysis (Ehrlich and Matsudaira, 1999), 3D organ printing (Mirinov et al, 2003), cell culturing (Gómez-Sjöberg et al, 2007) etc.

As microfluidics works on a smaller scale, it is important to understand the forces that dominate and how they can be used to manipulate and improve the performance of potential gas exchange devices. The principle design involves blood flow through an

array of micro channels, similar to capillary circulation of the lung. Simultaneously, gas exchange occurs across the surface of the membrane (Rochow et al, 2013).

It is important to note that different factors dominate the micro compared to the macro scale. These include high-pressure differentials, high surface tension relevant to bubble formation, high surface to volume ratio and laminar flow. Advantages include reduced cost per device, batch fabrication, improved performance, better control, faster results, and less material consumption (Vowel, 2009). As well microfluidics allows for dimensions much closer to those of the lung (capillary 5-10um), thereby maximizing blood phase contact to permeating gases along the surface of the membrane (Lee et al, 2008).

Initial attempts included creating devices for cell culture growth (Lam et al, 2009). Subsequently Hoganson et al (2010), reported the development of a microfluidic lung assist device whose design was based on achieving physiological shear stress and high gas exchange. The device achieved physiologic blood flow, and shear stress thereby minimizing platelet activation and thrombus formation. The O_2 and CO_2 gas exchange was 89.3 and 78.1 mL/min/m^2 at 4mL/min blood flow and increased with increasing flow rate. A disadvantage was that multiple layers would be needed to provide respiratory support necessitating the device to be extremely large (Hoganson et al, 2010).

Another similar devices have been reported (Potakay et al, 2011), (Hoganson et al, 2011), (Kniazeva et al, 2012), however despite their performance, large number of the

same microfluidic devices in a layered fashion are needed to provide support making them unfeasible for clinical use. However, their relatively low priming volume and dimensions that closely mimic lung dimensions making them an attractive option for the neonatal population. The limitations of current ECMO modalities could theoretically be overcome with a new form of oxygenator application, historically referred to as the "Artificial Placenta". Its history, theory and development are discussed in Chapter 3.

CHAPTER 3

ARTIFICIAL PLACENTA

3.1 Background

The 'Artificial Placenta' concept arose from early experiments attempting to maintain previable fetuses (Ernhorning et al, 1954; Westin et al, 1958). The artificial placenta's primary function is to provide respiratory support for neonates (term and pre term) via attachment post-natally to the umbilical vessels, thereby creating a partial fetal circulation and avoiding any need for surgical access to the central vessels as for ECMO. This would also reduce the risk of micro emboli to the brain. Blood would pass down the aorta toward the umbilical artery, into the artificial placenta device where O_2 would diffuse into the blood and CO_2 would be removed (Rochow et al, 2013). Subsequently the blood would be returned via the umbilical vein leading back into the ductus venosus. The ideal artificial placenta device would:

- Be pumpless because the baby would use its own heart to drive the blood flow into the device
- Be characterized by a high gas exchange
- Achieve sufficient extracorporeal flow
- Demonstrate low resistance
- Have a low priming volume
- Present as a haemocompatible device

- Be modular in design to accommodate varying body weights (Fig 3.1) (Rochow et al, 2013)

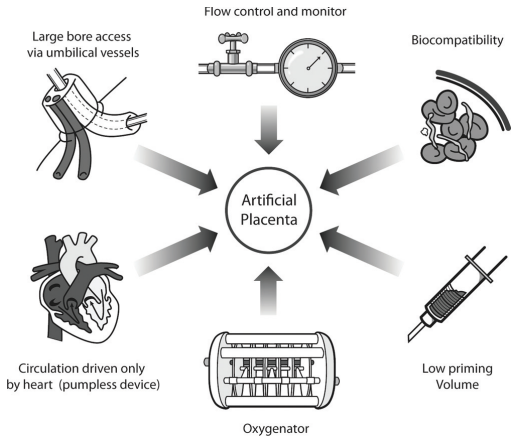


Figure 3.1 Characteristics of the artificial placenta (Rochow et al, 2013)

Early attempts at this concept began when Enhorning et al (1954), demonstrated that a single injection of oxygenated blood into the umbilical vein normalized the heart rate, blood pressure and primitive respiration of human foetuses (Enhorning et al, 1954). Subsequently, Westin et al (1958) attempted to perfuse human fetuses, with a system that included pumps and a spiral oxygenator. The fetuses were kept in an artificial intrauterine environment and pumps were used to reduce edema. Fetuses (n=7) were viable for 5-12 hours, and at flow rate of 14.2 mL/min via the umbilical vessels oxygen consumption of 7.6mL/kg/min was reported. Though pumpless extracorporeal flow was not achieved, this work paved the way for researchers to use different oxygenators as the artificial placenta device, which are reported in the following sections. Achievements and challenges are discussed.

3.2 Direct Oxygenators

Lawn and McCance (1962) successfully went on to create the first pumpless, heart driven extracorporeal flow via umbilical vessels in a piglet model. Pumps are undesirable as they lead to the need for additional priming volume, calibration and cause mechanical damage the blood cells. The 50 disc spinning oxygenator with total gas exchange area of 2500cm^2, and priming volume of 200-250mL achieved 1-4.0mL/kg/min of O_2 transfer with flow rates ranging from 5-20 mL/min. Fetal death occurred due to circulatory failure. The system was complicated due to the need to use a dialyzer, and humidifier to keep the gas exchanger at appropriate temperature (Lawn and McCance, 1962). Also the device was not feasible for long term use and its high priming volume made it unreasonable for clinical use in the infant population.

3.3 Indirect Coiled Membrane Oxygenators

In 1969, Zapol et al successfully supported premature lamb fetuses for 2 days using a silicone membrane oxygenator (Zapol et al, 1969). The device had a priming volume of 240mL, and gas exchange area of 0.4m^2. This setup was also not pump less, but higher flow rates and thus high gas exchange rates of 6mL/kg/min at flow rates of 70-150mL/kg/min were achieved, higher than for the Lawn and McCance, (1962) spinning oxygenator. However, Bui et al, (1992) reported complications of heart dilation and liver necrosis/ haemorrhaging associated with using coiled membrane oxygenators as artificial placenta. Awad et al (1995), attempted to use a low primed 90mL, coiled membrane

oxygenator (surface area 0.4m^2) however, they could not achieve a pump less configuration due to high resistance of device and to catheters impeding blood flow in a lamb model (Awad et al, 1995).

3.4 Hollow Fiber Oxygenator

Unno et al (1993), and Kuwabara et al (1989) both attempted to implement a silicone based non micro-porous membrane oxygenator (surface area 0.5m^2). They used pumps and had priming volumes of 200-230mL and 160mL for their respective system, which was lower than those, required for coiled and direct oxygenators as stated previously. Unno et al, achieved 80- 180mL/kg/min achieving O_2 transfer of 5-10mL/kg/min (correlating closely to reports by Zapol et al, 1969); and Kuwabara 93mL/kg/min with O_2 transfer of 6.6 mL/kg/min both using a fetal goat model. Unno et al, 1993, were able to achieve the longest perfusion time reported thus far of 494-542hours showing that long term extracorporeal support to the fetus is feasible and not harmful.

Yasufuku et al (1998), attempted to use a non micro porous polyolefin hollow fiber membrane, and maintained extracorporeal flow for 87-237hours. Using a centrifugal pump they achieved flow rates of 150-200mL/kg/min. Though no gas transfer data was reported they observed lung maturation and growth (Yasufuku et al, 1998). However, Sakata (1998) also used a non-micro porous polyolefin hollow fiber membrane and achieved flow rates of 113-193mL/kg/min with oxygen transfer of 4.4-5.3mL/min.

This device was vastly improved with respect to priming volume (95mL/min) relative to that of Unno et al (1993), and Kuwabara et al (1989). It is interesting to note the polyolefin membrane oxygenator performed less than the non-micro porous silicone membrane oxygenator used in Unno et al (1993) setup at similar flow rates. This is not surprising, as silicone has been the main material used due to its high gas permeability

A strong push towards achieving the pump less artificial configuration came when Awad et al, (1995) successfully used a micro porous polypropylene hollow fiber (priming volume 90mL) along with a heat exchanger to support lambs (1.2-3kg body weight). Extracorporeal perfusions between 4-6 hours were maintained which is considerably less than Unno et al (1993), Kuwabara et al (1989) and Yasufuku (1998). However, this system was able to maintain good CO_2 elimination although flow rates decreased from 72.49mL/kg/min- 33.4mL/min/kg over the series of the experiment. Despite achieving a pump less configuration, inadequate flow and high resistance of the device limited oxygen transfer, arterial pressure, cannula and viscosity of the blood (Awad et al, 1995).

To overcome the problem of resistance and high priming volume A miniaturized hollow fiber system was reported by Arens et al (2011), with a priming volume of 12mL and surface area of $0.06m^2$. Achieving maximum flow rates of 120mL/min, the device was not haemocompatible and unacceptable to premature infants due to risk of cerebral hemorrhage. In vitro tests showed that $53mLO_2$/L blood and $47mLCO_2$/L blood was transferred at flow rates of 80mL/min with oxygen flow of 160mL/minute In vivo testing

achieved maximum flow rate of 33mL/kg/min, however experiment times were limited to 3 hours.

Only Awad et al (1995), Reoma et al (2009), and Arens et al (2013) were able to achieve the pumpless configuration. However the flow was strongly impeded by both resistance of the device and cannulas used to catheterize umbilical vessel resulting in relatively shorter perfusion times as well. Oxygenators past and present do not fully facilitate the artificial placenta concept either due to high priming volumes, the use of roller pumps, high resistance, inability to achieve appropriate extracorporeal flow and low gas transfer.

Therefore, our group designed a novel lung assist device whose design is based on the principles of microfluidics and would emulate the concept of the artificial placenta. In the following chapter, design parameters for this novel lung assist device are presented and discussed.

CHAPTER 4

A NOVEL LUNG ASSIST DEVICE- DESIGN

Our group previously published a report, on the development of a new lung assist device (LAD) that was composed of a stack of single microfluidic oxygenator units (SOU) (Rochow et al, 2012). The primary goal of this LAD is to maximize gas exchange and in this chapter we will discuss the design parameters for each component of the LAD in a bottom up manner.

4.1 Single Oxygenator Unit (SOU)

A SOU is composed of two functional components: (a) a micro vascular network and (b) a gas permeable membrane. It relies on ambient air to diffuse across the gas permeable membrane in order to improve the O_2 saturation of the blood. Fick's equation relates that in order to maximize diffusion of gas molecules one needs to (i) increase the surface area, (ii) decrease the thickness of the interface across which diffusion occurs, (iii) use materials with a high diffusion coefficient of the relevant gas and (iv) have a high pressure gradient along the interface. These parameters are exploited within the different functional components and design of the LAD.

4.1.1 Micro Vascular Network

Different designs for vascular networks as part of a lung assist device have been suggested in literature. Hoganson et al (2010) designed a branched vascular network with the principle goal of achieving physiological flow in order to minimize damage to the blood (Fig 4.1). The channel widths range from a few mm to 200um, with 58.3% of the vascular network involved in gas exchange. Their design also incorporated an alveolar chamber in which pure oxygen served as the source of O_2. Gas exchange rates achieved were similar to those found in commercial oxygenators. However, the resistance of the device was high, ranging up to 123.1mmHg at 8.0mL/min.

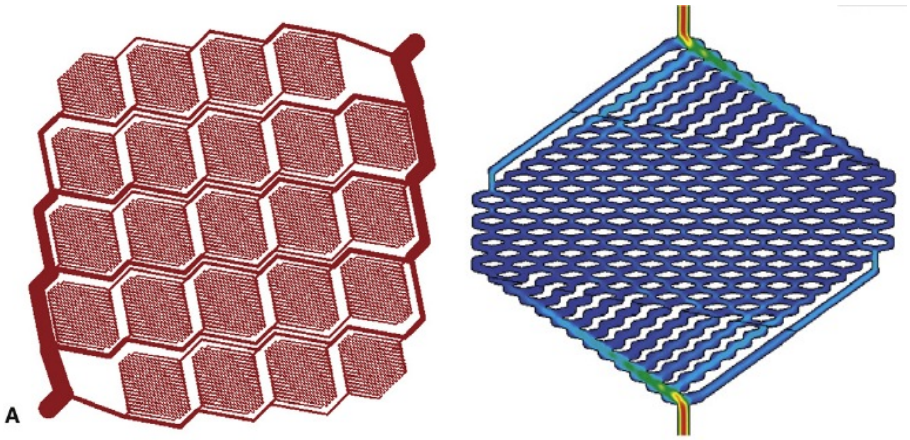


Figure 4.1 Micro vascular network design (Hoganson et al, 2010)

The same group (Hoganson et al, 2011) reported a second design of a micro vascular network designed to mimic physiological blood flow and shear stress. It had a

total surface area of 23.1cm^2 (Fig 4.2) with starting channel dimensions ranging from 748x 764um to 100x100um. However, the vascular channels only covered 30% of the device area, which was less than their previously reported design and limited the total available surface area for gas exchange. Kniezeva et al, (2012) also used a similar design intended to reduce disturbed flow by using smooth bifurcating channels with varying widths/ lengths, achieving uniform fluid distribution and wall shear stress.

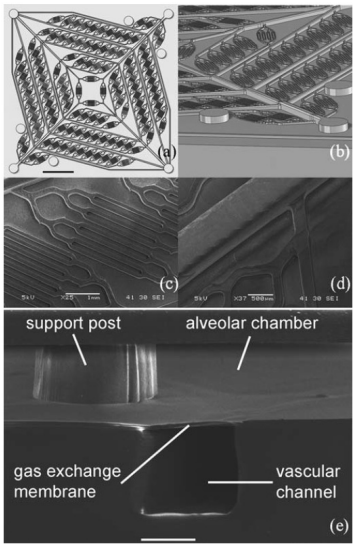


Figure 4.2 An example of micro vascular network design (Hoganson et al, 2011)

In 2009, Burgress et al micro fabricated an artificial lung device designed as an array of 56 long channels that were up to 1.8cm long. The vascular network was subsequently sandwiched between two alveolar chambers in an effort to improve oxygenation (Burgress et al, 2009). Each channel was 100um wide pitched at 50um. This device allowed for cultured cells to survive up to 10 days, however the disadvantage of

this design is that it does not take into consideration that blood flows in a laminar fashion at the micro level. Therefore to achieve optimal gas exchange, passive or active forms of mixing need to be induced in order to allow full saturation of the blood (Lim, 2006).

Finally, Potkay et al (2011) proposed a design that reached its goal of efficient gas exchange, however, its branched design did not achieve physiological flow characteristics, demonstrated high pressures, and fluidic shear stress. These are all important factors that contribute to blood coagulation.

The principal goal of our LAD is to maximize and facilitate efficient gas exchange. Therefore the SOU have been designed to maximize the functional surface area across which gas exchange occurs, as well as to minimize flow resistance and shear stress on the blood. The vascular network is square shaped, 43mm in width/ length. It consists of an inlet and outlet port. The blood flows through multiple micro channels (Fig 4.3) where each micro channel is 500um wide, and 80um tall with pillars pitched at 500um.

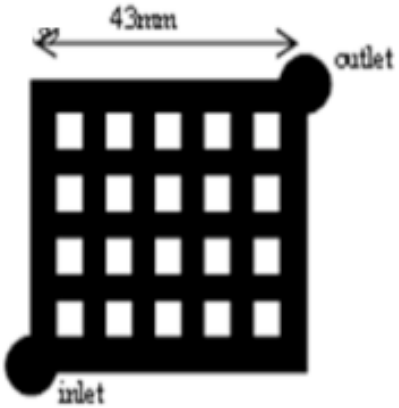


Figure 4.3 Our micro vascular network design

Though high gas exchange would be favoured with dimensions that are much closer to the lung, this factor must be counterbalanced with the ability to achieve channel uniformity, and reproduce devices with high reliability (Lee et al, 2008). Preliminary testing showed that channels of 500x500um allowed for sufficient bonding of the membrane without collapse or delamination during blood perfusion of channels.

Using COMSOL, a numerical simulation of; non Newtonian model, with Navier-Stokes equation, boundary set to non slip and the inner flow rate to 1mL and outer flow rate to 0 mL/min, various microfluidic heights ranging from 40-100um were examined. It was shown that 80um was the minimum height below which shear stress in the oxygenator did not surpass the blood coagulation threshold of 10 Pa (Kroll et al, 1996; Malek, 1999).

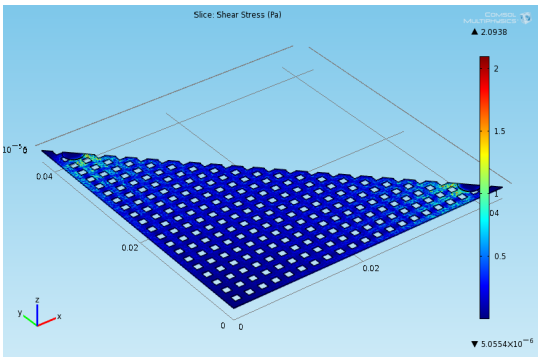


Figure 4.4 Shear stress modeling across SOU

Also with this dimension uniform velocity distribution was achieved, which is important for maximizing blood contact with the membrane and avoiding problems like

streaming which occurred in flat membrane oxygenators (Wegner, 1997). Even shear stress is achieved throughout the device Fig 4.4, (except for the inlet/ outlet), which is important as it can induce hemolysis releasing free haemoglobin into the blood (Lim, 2006).

The pillars function in providing mechanical support for the gas permeable membrane. At opposing corners of the micro vascular network the blood inlet and outlet are located, and this results in uniform flow distribution and minimal shear stress due to equidistant paths between the inlet and outlet. Under this design the total volume of the vascular network is 0.14mL per SOU. This design also ensures that 70% of the membrane area is available for gas exchange which is greater than previously reported designs (Hoganson et al, 2010; Hoganson et al, 2011).

The micro vascular network was molded using polydimethylsiloxane (PDMS). It is a polymer with an inorganic siloxane backbone and organic methyl groups attached to a silicone (Kuncová-Kallio and Kallio, 2006) as shown in Fig 4.5.

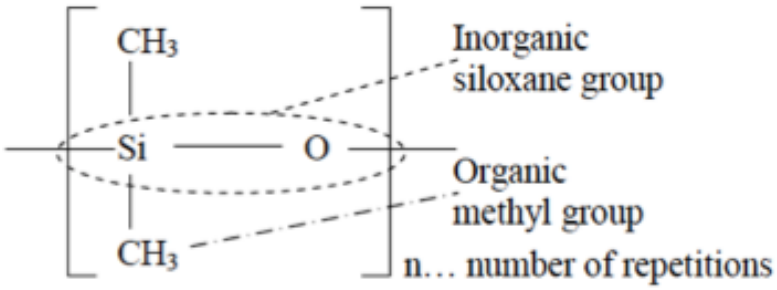


Figure 4.5 Structure of polydimethylsiloxane (Kuncova-Kallio and Kallio, 2006)

PDMS is inexpensive; it can be used a single time, conforms to most materials, withstanding temperature gradients and has a high gas permeability (Jessaimine, 2002) making it a popular choice of material for microfluidics (Kuncová-Kallio and Kallio, 2006).

4.1.2 Gas Permeable Membrane

The materials of choice for this interface are based on various criteria including permeability to O_2 and CO_2, mechanical strength and the ability to fabricate membranes without defects. Early membrane technology was based on hydrophilic materials, however it was soon realized that these were prone to leakage (Lim, 2006). The earliest oxygenators used teflon for mechanical strength. However it soon became apparent that hydrophobic materials were inferior in CO_2 diffusion (Lim, 2006). Clowes (1957) tested ethylcellulose, which showed good O_2 permeation; however it was mechanically brittle and difficult to fabricate membranes without pinholes. In an earlier paper Clowes reported on materials including polyethyelene, chlorinated rubber, mylar (polyester), polyvinyl chloride, saran, and cellophane and demonstrated that polyethylene was optimal with respect to gas permeation and no leakage (Clowes, 1955; Clowes, 1957). Eventually, porous polypropylene coated with a thin silicone layer became the material of choice for hollow fibers (Iwahashi, 2004) as silicone has one of the highest gas permeability's coefficients relative to other polymers especially with respect to greater diffusion of CO_2 vs. O_2 (Iwahashi, 2004; Mercel, 2000). Porous membranes soon followed which allowed

for intermittent direct contact between blood and gaseous phase, without the high blood trauma associated with bubble oxygenators (McCaughan et al, 1960).

The initial prototype of the LAD used a polycarbonate membrane due to its mechanical stability. The total functional membrane surface area of SOU was 1245mm^2 with a low priming volume of 8mL. The only two areas where the membrane lacks mechanical support in the form of pillars are above the inlet and outlet. Therefore, these areas are more prone to leakage as they are also the area of highest flow resistance with inlet diameter of 3mm and channels of 500um width. A simple clear tape (size 12.5 by 12.5mm) is placed above for reinforcement.

4.2 Lung Assist Device

The final LAD is a stack of parallel SOUs (Fig 4.6). The inlet and outlet are positioned at opposite ends and the LAD consists of 5 important parts. (i) The circular base (1cm thick and 10 cm diameter) (Fig 4.7) provides structural support and openings for the blood inlet/ outlet (diameter 3mm). It consists of four smaller holes (diameter 5mm) for connections of four support stands (Fig 4.6).

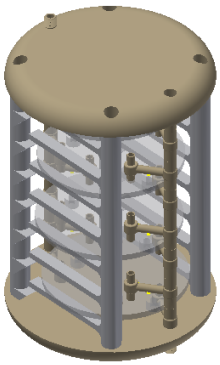


Figure 4.6 Full assembled LAD

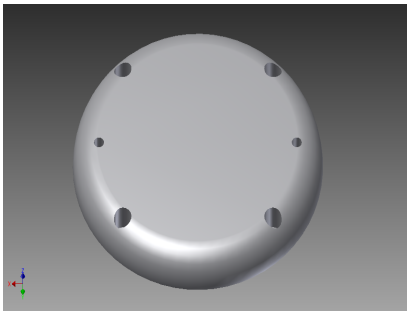


Figure 4.7 LAD base

The LAD's skeleton is a series of two types of T-connectors: (i) threaded and (ii) non threaded consisting of standard size #1/4-32. The threaded T connectors (Fig 4.8) connect the base to the skeleton of the LAD by placing to a threaded luer lock connectors on the opposing side (Fig 4.9).

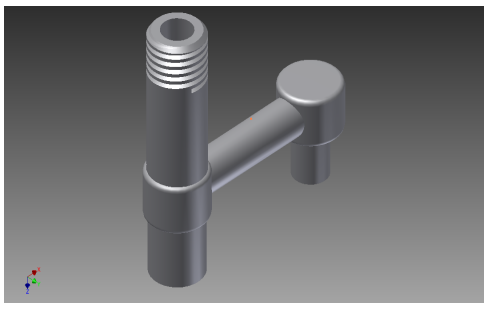


Figure 4.8 Threaded T- connector

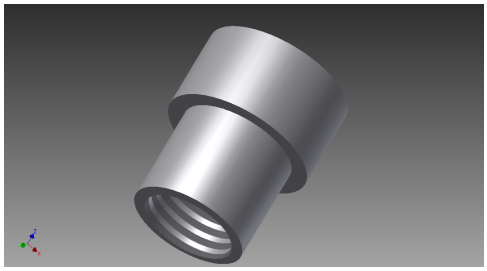


Figure 4.9 Luer lock connector

This stabilizes the bundle overall. One non threaded T- connectors has a length of 8mm, and inner width of 3mm (Fig 4.10).

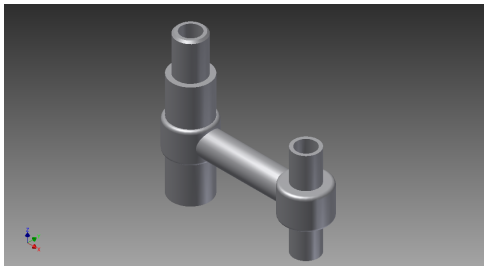


Figure 4.10 Non threaded T-connector

Two SOUs are paired by creating four rectangular openings around the outer edges, to allow for input of the opposing inlet/outlet SOU tubing (Fig 4.11). This type of assembly ensures that the membrane side is facing out towards ambient air allowing for gas exchange. Non threaded T- connectors are used to stack pairs of SOUs and assemble the final LAD.

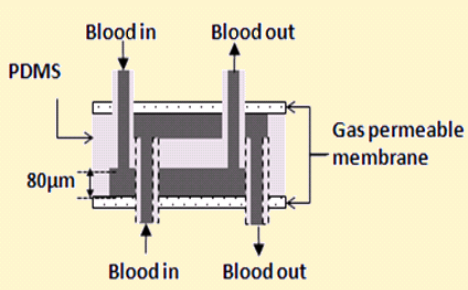


Figure 4.11 Back-to-back conformation of a pair of SOU

The LAD is equivalent in situation to an open ductus arteriosis, atrium/ ventricle septum defect and its design is based on the following theoretical considerations:

1. Blood flows through each SOU and is designed to raise the oxygen saturation of blood from 70% (typical in utero in the umbilical artery) to 100% (Rochow et al, 2013).

2. It is a pumpless design, and therefore flow is driven by the pressure difference between the umbilical artery and vein (30-40mmHg in premature infants) (Rochow et al, 2013).

3. It is assumed that 10% of the cardiac output volume extracorporeally would not lead to cardiovascular compromise (Takahasi, 1996) and is in the range that is needed to improve oxygenation of blood to physiological levels (Reoma, 2009). The following assumption was made for heart rate: 150 min^{-1}, stroke volume: 2

mL · kg^{-1} (body weight) (Agata, 1991); and extracorporeal shunt from cardiac output: 10 %. Extracorporeal bypass volume = heart rate · stroke volume · percent extracorporeal shunt = 30 mL · kg^{-1} · min^{-1} (Rochow et al, 2013).

Under these assumptions and based on experimental results obtained on PC membrane SOUs we deduce that 1 SOU is needed to support 100g body weight (Rochow et al, 2013).

CHAPTER 5

OBJECTIVES OF THESIS

1. In vitro: To optimize gas exchange transfer in the LAD by characterizing gas exchange properties of four different gas permeable membranes;

   a. Polycarbonate (0.1 um)

   b. Polycarbonate (0.05um)

   c. Normal PDMS membrane

   d. Porous PDMS

2. In vitro: Characterize the gas exchange of the full LAD under i) ambient air and (ii) oxygen rich atmospheric conditions.

3. In vivo: Assess feasibility of LAD in a newborn piglet model by:

   a. Studying the effects of extracorporeal flow rates on cardiovascular parameters via LAD attachment to the (i) umbilical and (ii) carotid/ jugular vessels.

   b. Characterize the gas exchange of LAD.

CHAPTER 6

FABRICATION

6.1 Micro Vascular Network

The micro vascular network in the body of the SOU is made using the soft lithography process, as described by Xia et al (1998). A silicon wafer was coated with photo resist and spun at 4000rpms to create the master. PDMS was prepared in 10:1 ratio of base: curing agent. The PDMS used was a 2-part kit: Sylgard TM 184, from Dow Corning consisting of a liquid silicon rubber base (a vinyl terminated PDMS) and a catalyst/ curing agent (a mixture of platinum, methylhydrosiloxane, and dimethylsiloxane). Silicone tubing was cut (inner diameter 3mm, length 10mm) and used as the inlet/outlet for the SOUs. Subsequently, 8mL of PDMS was cast on the master, and heated at 80°C for 1 hour. The liquid mixture undergoes cross-linking via the hydrosilylation reaction between vinyl ($SiCH=CH_2$) groups and hydrosilane (SiH) groups (Jessaimine et al, 1993). Once it is completely cured, the solid PDMS micro vascular network is cut out, peeled from the silicon master and the inlet/outlet are cleaned of any residual PDMS. The advantages of this technique are that it is inexpensive and easy to apply (Xia et al, 1998).

6.2 Gas Permeable Membrane

In order to determine the most effective interface for gas exchange the following gas permeable membranes were tested:

1. Porous polycarbonate (PC) membrane: Purchased from GE- Water (0.1 and 0.05um pore size). Both have a thickness of 6um, with pore densities of 4×10^8 and 6×10^8 pores per cm^2.

2. Normal PDMS membrane: PDMS was prepared in 10:1 ratio and 1mL of the solution was deposited on 80mm diameter parafilm substrate disc. Subsequently, the parafilm substrate was spun for 30sec to obtain the final microfilm.

3. Porous PDMS membrane: PDMS was prepared in 10:1 ratio. NaCl was grounded for 15 minutes and added to the PDMS to make a 5% NaCl PDMS mixture. 1mL of the solution was deposited on a parafilm substrate and spun for 30s to obtain the final microfilm. Subsequently, the parafilm was left at room temperature overnight to cure.

Burgress et al (2008) demonstrated that gas exchange was inversely related to the thickness of the membrane. Therefore, the minimum PDMS membrane thickness that we could fabricate, and was mechanically stable during fabrication was membranes of 25um thickness (spin rate: 4000rpms).

6.3 Assembly of final SOU device: Bonding between Micro Vascular Network and SOU body

This is an important step of the fabrication process as it determines the overall quality of the LAD. If the two components are not well bonded, the device is compromised putting potential experimental subjects at risk. Based on tests using a newborn piglet model, and measuring the resistance through the umbilical vessels at varying flow rates, we determined the LAD bonding needed to withstand a back pressure of 60mmHg.

Bonding of micro vascular network to gas permeable membrane was facilitated by surface activation using a low pressure oxygen plasma machine (60W, 30sec). Plasma treatment exposes surface silanol groups allowing for subsequent covalent bonding of the two components (Jessaimine et al, 2002). The two components were micro-contact printed together, using a PDMS mixture (10:1) as ink. The entire device was cured in an oven overnight at 40°C. After curing, the gas permeable membranes were released from membrane substrate.

6.4 Assembly of the LAD

Interconnects of the LAD were designed in Auto Desk Inventor. The subsequent CAD design was sent for 3D printing (ProJet™ HD 3000 printer; 3D SYSTEMS Corp.,

Rock Hill, USA) using VisiJet® EX200 Plastic. The connectors of the LAD skeleton were assembled and SOU's were paired in back to back fashion.

6.5 Challenges During Fabrication

6.5.1 Membrane Carrier

Initially a parafilm substrate was used to fabricate PDMS based gas permeable membranes. They are inexpensive, and easily available; however, the parafilm substrate was not suitable for high temperatures thereby prolonging the fabrication process by a full day. As well complete curing was not always obtained. In addition, parafilm had a tendency to curl at room temperature resulting in a pool of PDMS in the centre, and therefore giving an uneven membrane thickness, which could impact gas exchange and make the outer edge of the membranes more prone to leakage.

Therefore to overcome these limitations, teflon was used as an alternative substrate for membrane fabrication. Having a similar surface free energy to PDMS (20mN/m at 20°C), teflon substrate was reusable, thereby lowering long-term costs. As well teflon's planar surface resulted in uniform membrane thickness, and decreased fabrication time. Membranes were spun using a similar protocol as above, and cured at 40°C, for 3 hours. A disadvantage of teflon is that it collects dust very easily, so a simple wash with isopropanol is necessary. This is an important step, which if overlooked, leads to devices that are highly prone to leakage.

6.5.2 Poor Bonding

Low pressure oxygen plasma is quite prone to PDMS contamination, which can impact bonding between the two functional components of the SOU. This results in delamination, and leakage from the LAD, which is unacceptable in a clinical or animal setting. As well, the chamber size limits the number of devices that can be treated and requires a cleanroom facility (Eddings et al, 2008). Therefore, other technologies were explored.

"Air Plasma", is an industrial form of low pressure oxygen plasma, that uses room air to activate a variety of polymer surfaces (Plasma Treat, 2013). It consists of a robotic arm that holds a circular rotating nozzle, powered by 20000 volts. The nozzle is programmed at a specific height and rotational speed, which is positioned above the sample to be treated. To explore whether this technology would optimize SOU functionality, we determined whether this technology was feasible to use for our devices. Therefore, we tested (i) PC- PDMS, and PDMS-PDMS bonding with 2 different nozzle types (14 and 45 degree (2 inch wide nozzle)).

Preliminary testing showed that the PC membrane was mechanically unstable and deformed under treatment by air plasma. Therefore, this technology was not feasible for fabricating PC membrane SOUs. The 14 degree nozzle led to silicon dioxide formation on the PDMS surface, which prevented the formation of siloxane bonds.

The 2 inch nozzle activated the surface promoting PDMS-PDMS bonding. To determine the optimal setting for bonding of our SOUs, different treatment heights of: 6, 9, 12, and 18mm, (where height is defined as the distance of the treatment nozzle from the surface of the SOU), and treatment speeds of 100 and 300mm/s across surface were studied. To quantify PDMS surface activation after treatment with air plasma, a surface energy kit was used. The surface energy kit consisted of a series of alcohol inks, with specified known surface energy values. When the alcohol ink surface energy values matched with the treated PDMS sample value, the droplet spreads across the surface of the PDMS sample. Table 1, reports the surface energy activation of PDMS by air plasma treatment at various nozzle heights and speeds.

Height of Nozzle (mm)	Speed of Treatment across Surface (mm/s)	Surface Energy (mN/m)
6	100	>68
6	300	>68
9	100	>68
9	300	40
12	100	32- 34
12	300	<28
18	100	<28
18	300	<28

Table 1 Surface energy of PDMS activated by air plasma

With increasing treatment height of nozzle from sample surface, there was a corresponding decrease in the surface free energy. As well samples treated at 300mm/s had a lower surface free energy than samples treated at 100mm/s at the same height. To determine how this affected device performance, human blood (hematocrit 50%, 40mmol/L of sodium bicarbonate and 5IU/mL of heparin) was perfused through devices bonded at various treatment heights and nozzle speeds at flow of 1mL/min. Initial resistance, and resistance at which device leaked/ delaminated are shown in Fig 6.1.

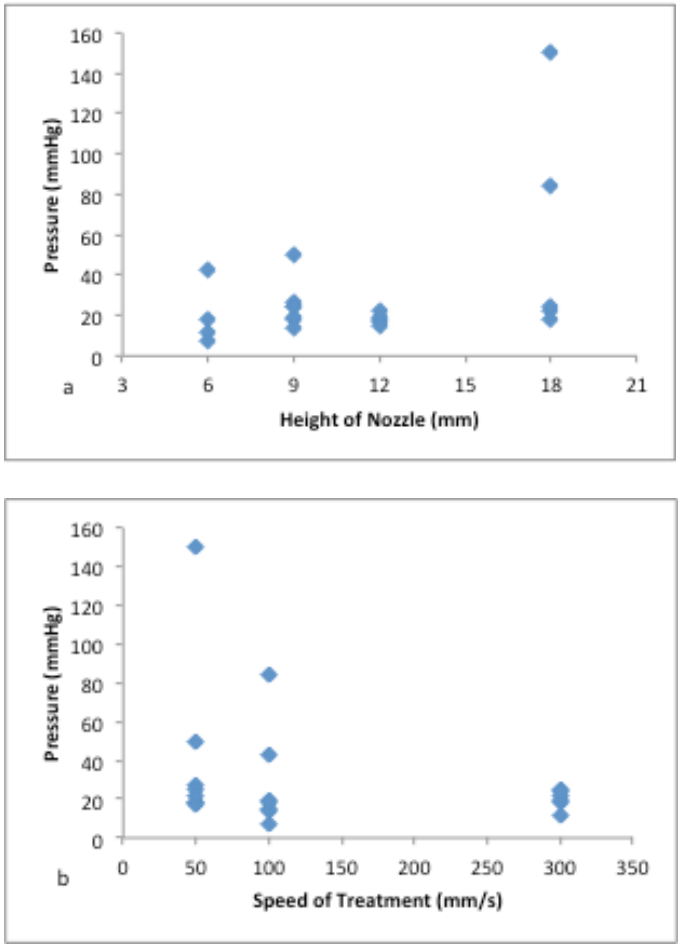


Figure 6.1 (a) Height of nozzle and (b) bonding speed, vs. oxygenator original resistance at flow rate of 1mL/min

The SOUs with highest initial resistance were seen at heights of 18mm and speeds of 100mm/s.

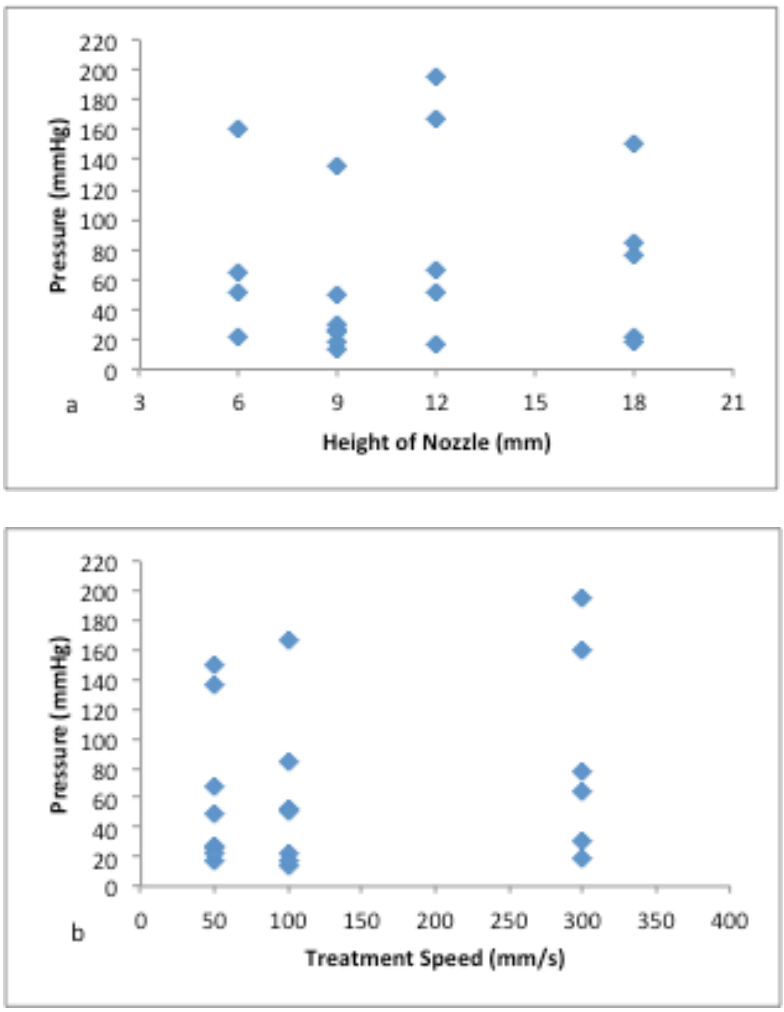


Figure 6.2 Pressure at leakage point of oxygenators bonded at varying (a) height of nozzle and (b) treatment speeds at flow rates of 1mL/min

No leakage was observed. Due to variability in data (Fig 6.2 a, b), an optimal setting for binding could not be identified. The oxygenators mean point of leakage was 62.55mmHg (range of 14-195mmHg). This was sufficient to support a 1kg piglet with a backpressure of 60 mmHg. Normal PDMS oxygenators bonded with low pressure oxygen

plasma machine and demonstrating strong bonding showed initial mean resistance of 44mmHg at flow rates of 1mL/min whereas air plasma machine bonded oxygenator have an overall mean resistance of 30.8mmHg at flow rates of 1mL/min. In a total of 20 devices, one device demonstrated delamination, 12 leaked at random pinpoints through the membrane while 7 leaked through the inlet/outlet. Leakage could be a result of multiple factors including defects in fabrication and not conducting air plasma treatment under cleanroom conditions.

Our preliminary testing with air plasma showed high potential, however, this was an extremely expensive technology. As well, tests were done on a small sample size due to limited access. Therefore further validation of results is needed.

Other inexpensive methods for bonding PDMS components are bonding of semi-cured devices, use of corona discharge (Eddings et al, 2008) and chemical adhesion using 3-Aminopropyltriethoxysilane **(**APTES) (Aran et al, 2009). Attempting to bond semi cured components leads to blocked channels and difficulty in releasing PDMS membranes from its carrier. As well, PDMS is transparent and it was very difficult to determine when the device was completely cured. APTES is an alternative suggested technique however, this technique makes PC membrane handling difficult and leads to channel formation towards the outer edges. As well, any form of chemical contamination can put our animal experiments at risk. In addition the bond was destroyed by water, and over the long term damaged PC membranes. Corona discharge is another alternative,

however literature reports that the bond created is equal in strength to low pressure oxygen plasma (Eddings et al, 2008).

It was concluded that air plasma technology looked the most promising with respect to strong bonding, however it is an expensive machine. To alleviate the issue of poor bonding, we purchased a new low pressure oxygen plasma machine, and implemented an isopropanol cleaning method for the chamber.

CHAPTER 7

IN VITRO GAS EXCHANGE TEST

7.1 Objectives

1. To determine the best interface for gas exchange by studying the properties of four different types of gas permeable membranes (PC 0.1 and 0.05um, Normal PDMS and Porous PDMS).
2. To assess the gas exchange performance of the LAD under (i) ambient air and (ii) oxygen rich atmospheric conditions.

7.2 Experimental Setup

McMaster University Animal Research Ethics Board approval was obtained prior to commencement of the study. Human blood was prepared to a hematocrit of 50% with plasma, and adjusted to a sodium bicarbonate concentration 40mmmol/L and 5IU heparin/L for anticoagulation. Blood was pumped via a peristaltic pump into a commercial hollow fibre oxygenator (OXR® Living Systems Instrumentation, Burlington, VT, USA) into a closed beaker, where the O_2 and CO_2 was adjusted to mimic hypoxic conditions (oxygen saturation value and partial pressure of O_2 and CO_2 (9 ± 5 %, 11 ± 7 mmHg and 54 ± 11 mmHg). Subsequently the blood was pumped into (1) the different membrane SOUs at varying flow rates (0.5-4.0mL/min) where a commercial oxygenator was used as a control. (2) A LAD with 10 SOU was tested at varying flow rates (5-

40mL/min) under (i) ambient air conditions and (ii) oxygen rich atmosphere. Oxygen rich atmosphere was created by boxing the LAD and flushing pure oxygen at a rate of 15L/min. Pressure as a reflection of resistance was recorded at varying flow rates by a pressure transducer (TruWave Pressure Transducer (Edwards Lifesciences LLC, Irvine, CA, USA) connected to a Spacelabs 90369 Patient Monitor (SpaceLabs Medical Inc., Redmond, WA, USA). A pre and post SOU, blood samples was taken and the gases were analyzed using a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Lecington, MA, USA). From this data we calculated the total O_2 and CO_2 dissolved in the plasma and erythrocyte fluid (See Appendix A) (Fig 7.1) (Rochow et al, 2013).

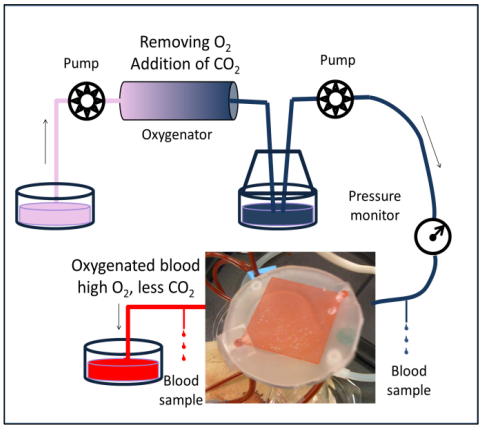
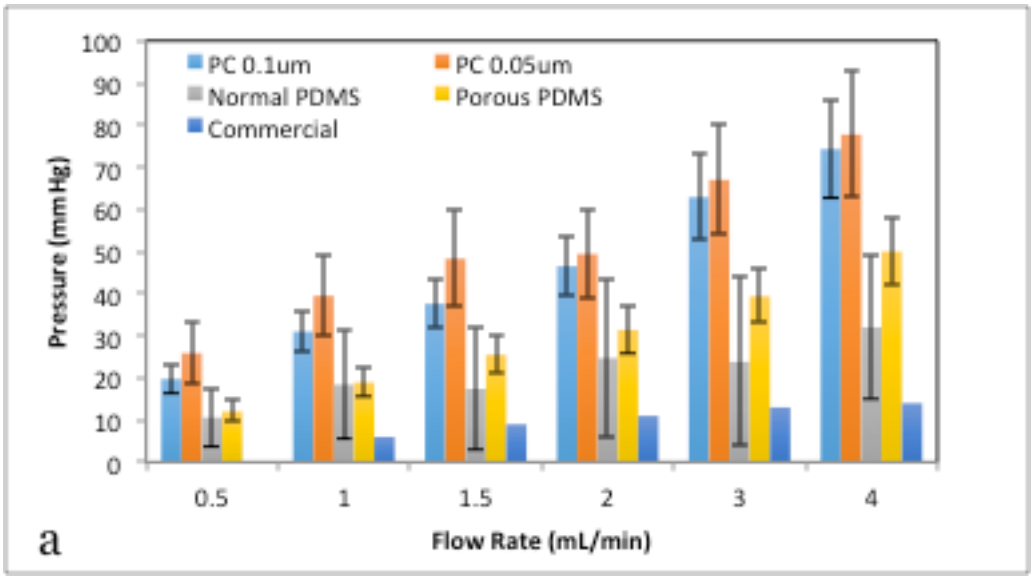


Figure 7.1 Experimental setup for gas exchange testing of SOU and LAD

7.2 Result

7.3.1 Objective #1

With increasing flow rate the O_2 and CO_2 transfer increases (Fig 7.2 a,b,c). Relative to the commercial oxygenator, all SOU types performed ~ 2.4 x and 1.3x better with respect to O_2 and CO_2 gas exchange. Both PDMS membranes demonstrated a lower resistance than PC. However, the resistance of the commercial oxygenator was significantly lower (~1.4x) than all SOUs. CO_2 transfer was significantly higher than O_2 transfer across all SOU types.



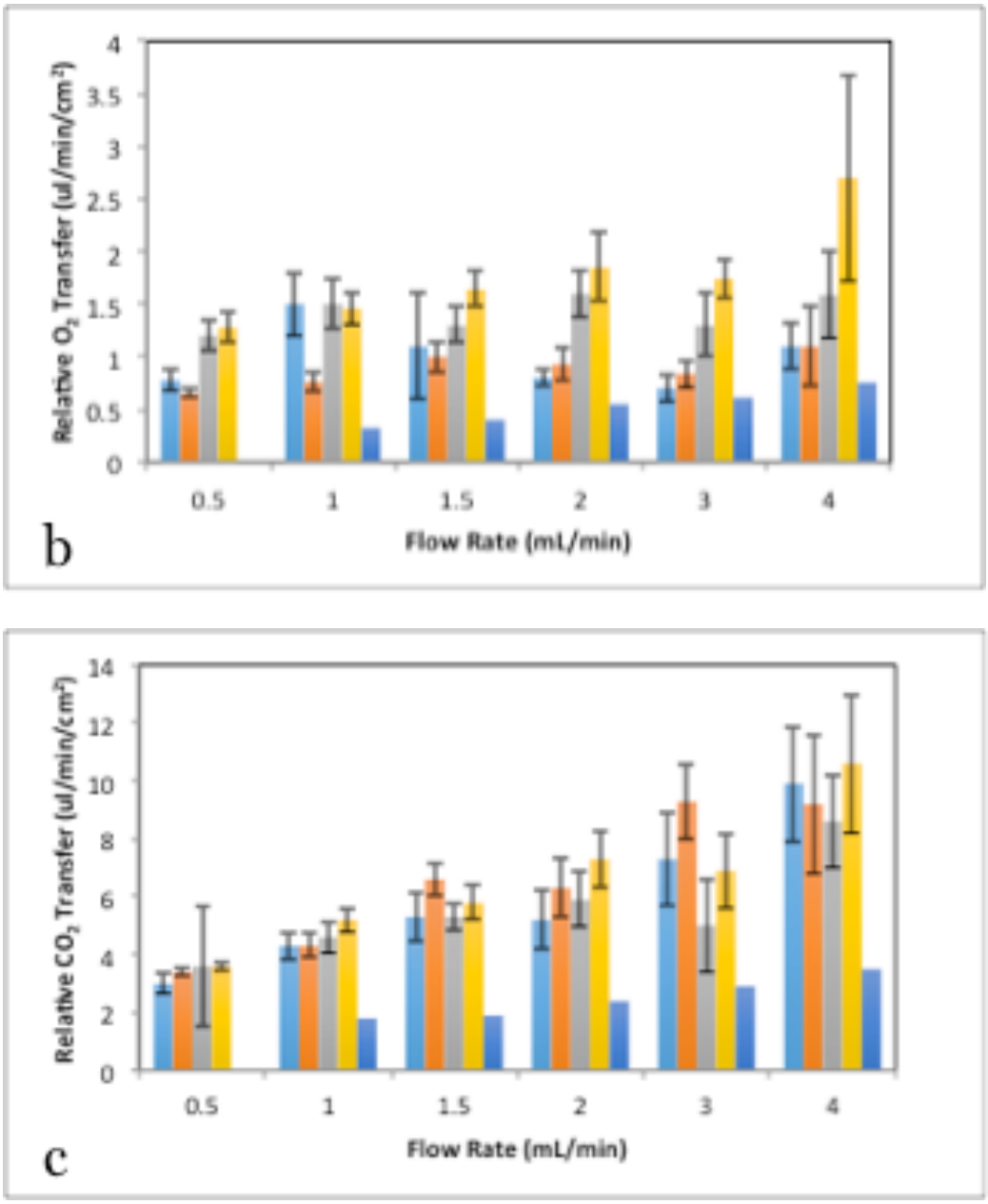


Figure 7.2 (a, b, c) Pressure, O_2 and CO_2 of different gas permeable membranes SOU and commercial oxygenator

The PC (0.1um) membrane performed the least efficiently with respect to O_2 exchange, followed by the PC (0.05um) membrane. Porous PDMS had the highest gas

exchange rate of 1.46 μL/min·cm^2 for O_2 and 5.27 μL/min·cm^2 for CO_2 and was 1.3 and 1.8x higher with respect to average O_2 transfer than normal PDMS, and PC 0.1um (Fig 7.2b,c). The normal PDMS membrane performed 1.4x greater than PC 0.1 um.

7.3.2 Objective #2

In vitro experiments (n=7) were performed using a normal PDMS LAD and tested under ambient air and oxygen rich air atmosphere. Based on the assumption that 1SOU would support 100g bodyweight, an LAD of 10 SOU designed to support 1000g body weight was assembled. With increasing flow rate there is an increase in resistance, O_2 and CO_2 transfer under both ambient and oxygen rich atmosphere (7.3 a-d). The average O_2 exchange under ambient air was 2.4 μL/min/cm² compared with 3.8 μL/min/cm² in oxygen rich atmosphere, resulting in an improvement of 1.6x (Fig 7.3 a, c). The average CO_2 gas exchange under ambient air and oxygen rich atmosphere was 10.1 μL/min/cm² and 6.4 μL/min/cm² respectively (Fig 7.3 b, d). The total run time of LAD was 5 hours.

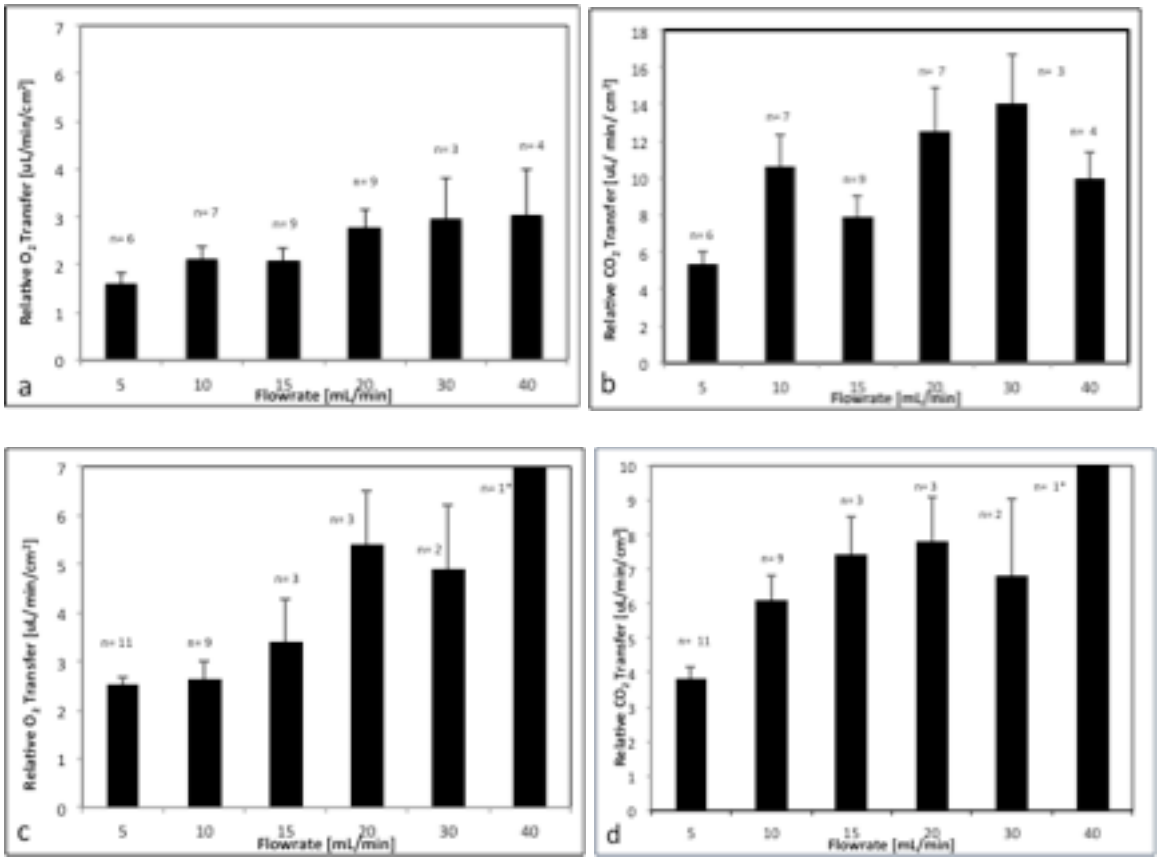


Figure 7.3 In vitro test: O_2 exchange rate at various flow rates under (a) ambient air and (b) O_2 rich atmosphere. CO_2 exchange rate under various flow rates under (c) ambient air, and (d) O_2 rich atmosphere. * Values above axis: O_2 and CO_2 (10.4ul/min/cm^2)

The average pressure of the LAD increased linearly with flow rate ranging from 20 to 61.5 mmHg as shown in Fig 7.4.

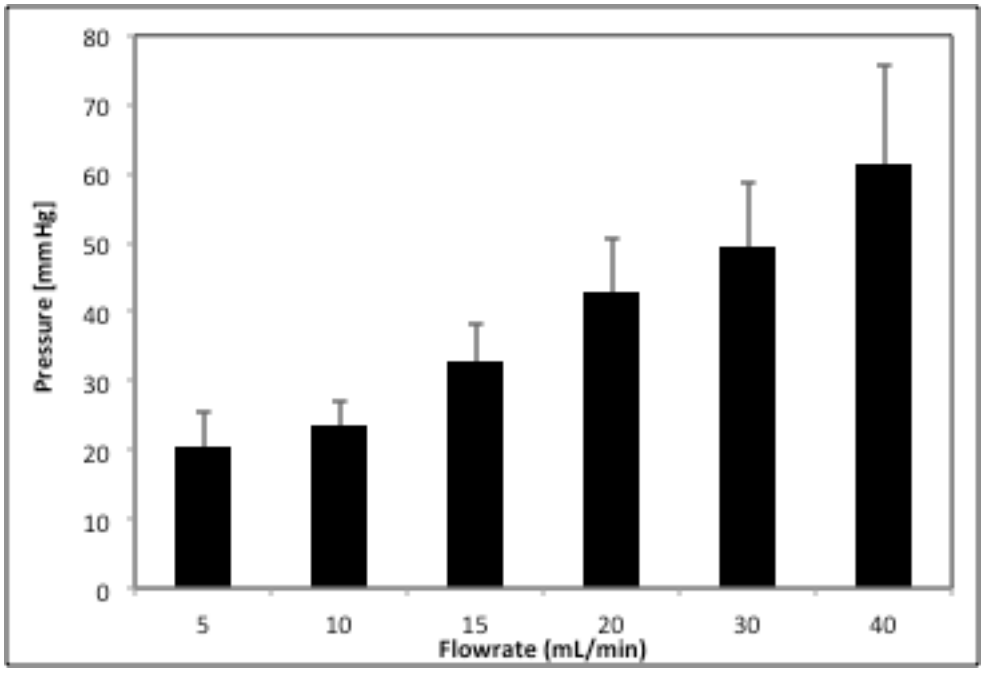


Figure 7.4 Average pressure of LAD against varying flow rates

7.4 Discussion

7.4.1 Objective #1

With increasing flow rates, there is an increase in both O_2 and CO_2 gas transfer across all SOUs (Fig 7.2 b, c). Similar to reports by Hoganson et al, 2010, this is expected, as with greater flow rates, there is a greater volume of blood and thus haemoglobin available for diffusion of O_2 and CO_2. CO_2 exchange is higher than O_2 and this is due to higher permeability of PDMS to CO_2 than O_2 (Merkel et al, 2000). The O_2 transfer appears to increase linearly at a lower rate when compared to CO_2 exchange. This can be explained by the direction of diffusion gradients. O_2 diffuses towards a small volume of haemoglobin, found within the microfluidic chamber thereby reaching a

maximum potential, where the flux of permeating O_2 will match the uptake of O_2 by haemoglobin. However, CO_2 diffuses towards a larger volume i.e. open ambient air and we can expect that with even higher flow rates it will continue to increase.

The PDMS membranes performed significantly better with respect to gas exchange than PC due to its high gas permeability coefficient. The porous PDMS was 1.3x higher with respect to O_2 transfer than normal PDMS, and this is related to the pores created by the sodium chloride crystals during the fabrication process. Even though the pores are not open pores, they provide a smaller diffusion distance to gas, thereby performing better than the normal PDMS SOUs. All of the SOUs outperformed the commercial oxygenator with porous PDMS membrane having the highest gas exchange rate of 1.46 $\mu L/min \cdot cm^2$ for O_2 and 5.27 $\mu L/min \cdot cm^2$ for CO_2. Based on this the porous SOUs outperformed the commercial oxygenator by 367% and 233% respectively.

Our device gas exchange performance is relatively lower than Hoganson et al (2010) by a factor of 5.6 with respect to O_2 transfer while we achieved slightly higher CO_2 transfer respectively at similar flow rates. However, their device contained an alveolar chamber, flowing pure O_2 above the vascular network as opposed to ambient air used in our experimental setup. Compared to Hoganson et al (2011) device design, we achieved similar gas transfer in terms of O_2 and CO_2, despite their addition of an alveolar chamber and using a membrane that was 3 times thinner than ours. Potkay et al (2011) achieved 15 $ul/min/cm^2$ and $26ul/min/cm^2$ of O_2 and CO_2 transfer which is 10X and 6X

greater than ours; however, their dimensions more closely mimic the natural lung capillaries with channel heights of 10 and 20um, width 88mm, resulting in an increased surface to volume ratio. In addition their PDMS membrane was 10um thinner than our design, which reduced diffusion distance for gas. However, their resistance was significantly higher than ours, which is undesirable, and the vascular network designed produced uneven shear stress, increasing the risk of thrombus formation (Potkay et al, 2011).

With respect to resistance the PDMS membranes had a lower resistance overall than PC membranes. This is due to the elastic nature of PDMS as PC is quite rigid and cannot accommodate the increase in flow. All SOUs demonstrated a higher resistance than the commercial oxygenator, however, we presume that when multiple SOUs are placed in parallel the resistance will decrease. Our O_2 and CO_2 data indicates that a PDMS membrane is the most optimal interface for gas exchange in the LAD.

7.4.2 Objective #2

One layered normal PDMS membrane demonstrated leakage when assembled into a LAD. We hypothesized that this was due to high variation in resistance across SOUs in the LAD. Therefore, double membranes SOUs were used for in vitro and subsequent in vivo testing of the LAD. Similar to the previous experiment, the exchange of CO_2 was higher than the O_2 exchange. This is explained by the fact that PDMS has a greater

permeability to CO_2 than O_2 (Merkel et al. 2000). As well the gas transfer rates increased with higher flow rates due to greater availability of haemoglobin under both ambient air and oxygen rich conditions. The LAD performance was 2.4 and 10.1 μl/min/cm^2 of O_2 and CO_2 under ambient conditions. In comparison, under oxygen rich atmosphere the LAD demonstrated 6.4 μl/min/cm^2 of O_2 and CO_2 transfer. The O_2 transfer increased by a factor of 1.6 between the two settings indicating that a change in LAD's setting can optimize gas exchange.

In LAD's current design the membrane area for gas exchange per kilogram body weight is ~ 130 cm^2 and assuming that in a healthy low birth weight infant the O_2 and CO_2 production is 4.7 ± 0.5 mL/kg/min and 4.5 ± 0.4 mL/kg/min (Bauer, 2009), theoretically our LAD could support 6-11% and 18-29% of O_2 support and CO_2 respectively. Pressure data (range: 20-60mmHg) is similar in range to mean blood pressure of 1 week-old pre term and term neonates (Pejovic et al. 2007, Kent et al. 2009). Therefore, neonates with lower blood pressure will not achieve sufficient gas exchange due to inability to achieve appropriate extracorporeal flow.

CHAPTER 8

IN VIVO EXPERIMENTS

8.1 Objectives

3a) Study the effects of partial extracorporeal flow through (i) umbilical and (ii) carotid/ jugular vessels on cardiovascular parameters.

b) Assess O_2 transfer in LAD via attachment to the umbilical vessels

8.2 Methods

The LAD was tested in a newborn piglet model (Strain Yorkshire). To ensure quality control, the LAD was initially perfused in vitro with isopropanol for 15 minutes, followed by saline flush for 15 minutes to observe for any leakage. The experimental setting for the in vivo study was adopted from Schmidt et al. 2004. The piglet was anesthetized with an initial dose of intra-peritoneal sodium pentobarbital (30 mg/kg). Subsequently, a 22 gauge angio-catheter was inserted into an ear vein placed for a maintenance infusion of Dextrose (80mg/kg/d) as nutrient supply and maintenance infusion of sodium pentobarbital (16mg/kg). Tracheostomy was performed using endotracheal 3.5 tubes and connected to a mechanical ventilator. Initial ventilator settings were chosen to ensure physiologic gas exchange: peak inspiratory pressure (PIP) of 1.5 kPa (15 cm H2O), positive end-expiratory pressure (PEEP) of 0.5 kPa (5 cm H2O),

inspiratory time (IT) of 0.5 s, respiratory rate (RR) of 30 breaths/min. A humidified gas mixture (warmed to 38° C) of 30% oxygen in nitrogen was delivered at 8 L/min. An ISC probe was placed on abdomen to monitor body temperature at 39C. For systemic blood pressure measurement a 3.5 Fr Argyle umbilical catheter (Sherwood, Medical,St. Louis) was inserted into the right femoral artery. Heart rate was also recorded. For anticoagulation heparin was given into the extracorporeal circuit starting with a bolus of 400 units/kg of heparin and maintained with heparin infusion rate of 30 units/kg/h.

Two different type of extracorporeal bypass were created by connecting the LAD to i) umbilical artery and the umbilical vein using customized single lumen polyurethane umbilical catheters as shown in Fig 8.1a(3.5 Fr and 6.5 Fr shortened to 10 and 6 cm)(Utah Medical Products, Inc., Midvale, UT, USA). 2) The left carotid artery and right jugular vein were accessed by a 14 Gauge 1.1” length angio-catheter as shown in Fig 8.1b(BD Catheters). Connection to a flow sensor was established and pre and post LAD blood samples were collected and analyzed for gas exchange using a Gem Premiere 3000.

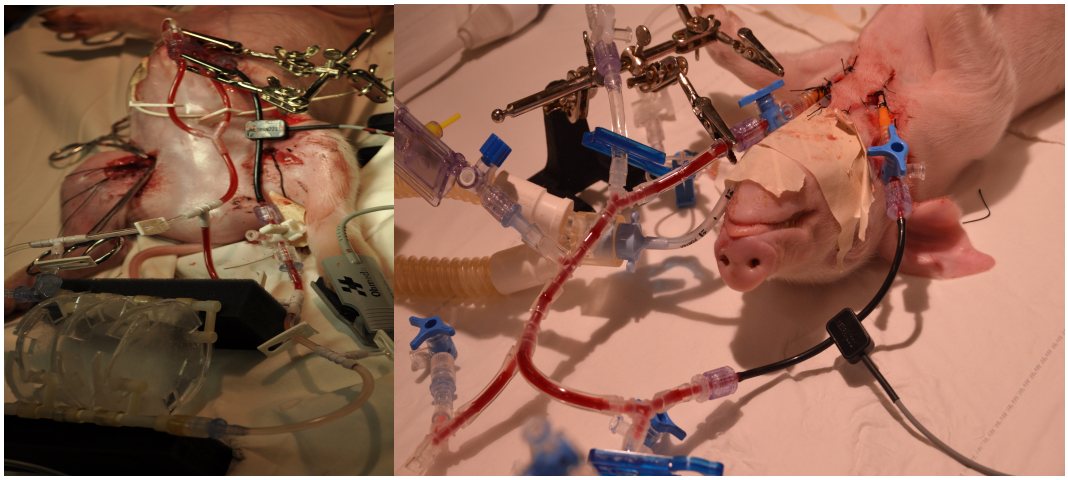


Figure 8.1 Extracorporeal flow accessed via (a) umbilical and (b) carotid/ jugular vessels

8.3 Results

8.3.1 Objectives #1

A total of 11 experiments were conducted with newborn piglets (body weights 1.43-2.1kg). Two animals experiments failed due to rupturing of the vessels leading to internal bleeding of the piglet. Of the remaining nine experiments, n=2 experiments were conducted to assess effects of extracorporeal bypass flow through the umbilical vessel while n=7 were used to study flow through the jugular/ carotid access.

(i) Umbilical vessel access: A maximum flow rate of up to 10.4mL/kg/min (n=2) was achieved while maintaining stable heart rate and blood pressure without administration of medicine to stabilize blood pressure. In one experiments, the piglet died before attachment of LAD. Cause of death was loss of blood volume.

(ii) Carotid/ jugular access: In n=5 experiments a maximum flow rate of up to 61.9mL/kg/min was achieved with a heart rate of 218bpm and mean blood pressure of 84mmHg as seen in Fig 8.2(a, b). Two flow experiments were conducted at two different time set on Piglet #4 (A and B). Two experiments were excluded; in one experiment flow and cardiovascular parameters were biased due to infusion of Solmnotol, which changes the heart rate and blood pressures. In the second experiment, no extracorporeal flow data was recorded.

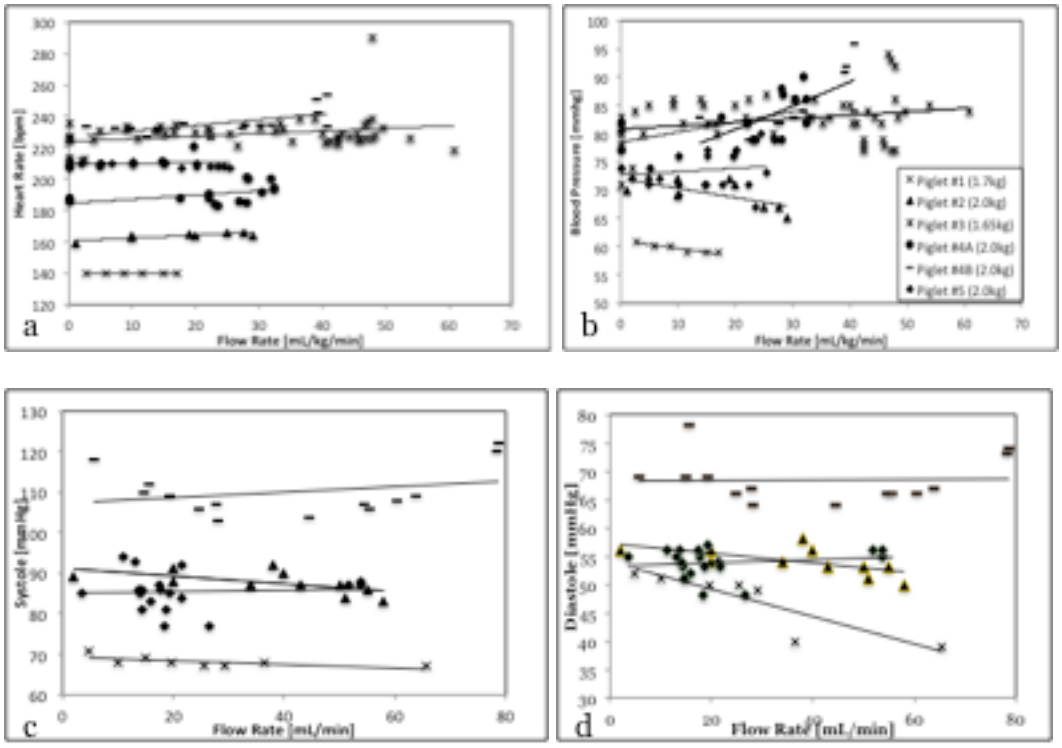


Figure 8.2 Effect of extracorporeal bypass of systemic circulation via carotid artery and jugular vein on cardiovascular parameter (a) heart rate (b) blood pressure (c) systolic pressure and (d) diastolic pressure

Heart rate remains stable as indicated by relatively flat trend lines while there are insignificant small increases/ decrease in blood pressure (Fig 8.2a,b). This is further emphasized when looking more closely at systolic/diastolic pressures, showing insignificant changes with increasing flow rates (Fig 8.2c,d). Piglet #3, and #4 are not shown on Fig 8.2d and 8.2e, as only mean blood pressure was recorded. Piglet #6, showed decrease in diastolic pressure; however, the last two data points were recorded when the piglet was being connected to the LAD.

8.3.1 Objective #2

An LAD with n=6 SOUs was successfully attached to the umbilical vessels. The table below shows the pre-post gas exchange that occurred at a flow of 13.5 mL/min with n=3 blood samples.

Relative Oxygen transfer (uL/min/cm^2)	**Pre- LAD Oxygen Saturation Value (%)**	**Post- LAD Oxygen Saturation Value (%)**
2.39	81	91
2.55	80	84
4.15	60	86

Table 2 Relative O_2 transfer through LAD (n=6 SOUs) connected to the umbilical vessels

At a flow rate of 13.5mL/min gas exchange of oxygen for the LAD was on average of 3.0 μL/min/cm^2 under hypoxic conditions (FiO_2 0.15) raising the oxygen saturation value from 74 to 87% (Table 2). The mean arterial blood pressure was 88 mmHg, with a heart rate of 253 bpm. The total duration time of the LAD was 30 minutes.

8.4 Discussion

Through the umbilical vessels (n=2) we achieved a maximum flow of 10.4mL/min/kg, which is relatively lower than that achieved through carotid/ jugular access. The umbilical vessels shrink after birth, thus limiting blood flow and gas exchange transfer. It also increases cannulation time, as vessels are difficult to access; indicating a need to improve vascular access which has been previously noted in literature as a challenge (Awad et al, 1995).

Preliminary testing showed that our LAD (n=6 SOUs) achieved gas exchange of 3.0 ul/min/cm^2 of O_2 at flow rates of 13.5mL/min via the umbilical vessels, improving oxygen saturation from 74 to 87%. When compared to in vitro gas exchange results of LAD at a flow of 10mL/ min, the average O_2 transfer is 2.1 ul/min/cm^2 indicating little change in O_2 transfer rates between the two different experimental settings. However, the CO_2 values cannot be compared to the in vivo setting because in the in vivo system, the piglet lungs are completely healthy. Therefore, they are still functioning to remove the CO_2 from the blood, thereby decreasing the rate at which it occurs through the device.

From these results if we were to assume that O_2 and CO_2 consumption is 4.7 mL//kg/min for pre term neonates, our calculations demonstrate that we can successfully support 7% of O_2 consumption similar to the 6% observed during in vitro testing of LAD under ambient air conditions. We can further improve this, by placing the LAD under an oxygen rich atmosphere. It is also important to note that newborn piglets average a haemoglobin count of 78.7g/L for piglets <1000g (Dale et al, 1967) which is approximately 3x lower than human neonates (Jopling et al, 2009). Our piglets in this experiment averaged 67g/L haemoglobin. Therefore, gas exchange transfer in our LAD may be higher under human neonatal blood conditions, due to greater transport capacity of the blood, having greater clinical implications than what we have reported here. Extracorporeal bypass was achieved without having the newborn piglet decompensate, therefore indicating that our device is feasible to use in a clinical setting.

The experiments have short run time due to delamination between the vascular network and gas permeable membrane in individual SOU's within the LAD. The first probable reason for this is poor bonding. However, the LAD in the in vitro setting run for up to 5 hours, without presenting delamination indicating a strong bonding between the gas permeable membrane and vascular network. Therefore, a more likely reason is pulsatile flow within the in vivo setting as a result of the heart being a 2-way valve which we have not mimicked within the in vitro setting, where we use a non pulsatile roller pump. Another factor also is that uneven perfusion, due to uneven resistance across SOUs could lead to some SOUs being more prone to delamination then others. To distribute

pressure evenly through the LAD, we modified the setup by assembling 3 LADs with 6 SOUs per each LAD, for a total of 18 SOUs. As well to reduce the effects of pulsatile flow, we modified the setup by adding a water trap prior to the oxygenator to act as a pulse damper thereby reducing the effects.

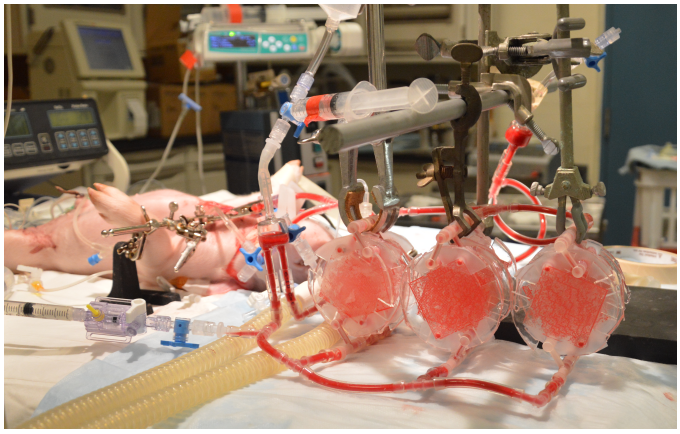


Figure 8.3 Modified setup to adjust for pulsatile flow adn varyign resistance across device

Three SOUs still delaminated in the LAD, after a few minutes of perfusion (flow rate 30mL/min). However, this new setup allowed for even flow through all devices. Blood samples were extracted for gas exchange and demonstrated an average O_2 transfer of 3.5ul/min/cm^2 at a flow of 14 mL/min, similar to that achieved via the umbilical vessels (n=5 blood samples). Prior to attachment of the water trap, the systolic, diastolic difference through the extracorporeal circuit across two piglet #6, and #7(2.0 and 2.1 kg) was an average of 30 and 35mmHg. Post water trap attachment to the circuit, the systolic-diastolic difference reduced to 3 and 0mmHg with a maximum flow of 12.6mL/kg/min. These results are preliminary and based on a small sample of n=1. Further, experiments need to be repeated to validate the results

CHAPTER 9

CONTRIBUTIONS/ FUTURE WORK

9.1 Contributions

Our group has developed a novel LAD that is composed of a stack of SOUs and has a total low priming of 8mL. In this thesis it was demonstrated that PDMS based membranes is efficient compared to other gas permeable membrane types for optimal gas exchange in the LAD. The LAD can provide between 6-11% and 18-26% of O_2 and CO_2 of neonatal consumption, when placed under ambient air and oxygen rich atmospheric conditions respectively. Pump less configuration of the device was achieved as the newborn piglet used its own heart to achieve extracorporeal flow of up to 60.9mL/kg/min without any compromise in cardiovascular parameters. As well preliminary in vivo gas exchange data of LAD match closely to those observed through in vitro testing.

9.2 Future Work

The artificial placenta concept appears promising however further work is needed to optimize the device. The main problem in conducting the experiments is high resistance of both the device and limitation of catheters to create a large bore access needed to create high extracorporeal flow. In addition, the gas exchange properties of the LAD could be optimized by using thinner membranes or membranes of greater porosity.

However, for porous PDMS oxygenators these factors must be balanced, with ease of fabrication, uniformity, repeatability and quality. Thin membranes are also difficult to handle, and have easier propensity to collapse within the channels.

Alternative methods to increase the membrane area is to have a new design where we increase the gas exchange across the body of the SOU. Smaller dimension which are much closer to those of the lung can also increase the surface to volume ratio resulting in improved gas transfer. However, this becomes a tradeoff with increasing resistance. As well, the LAD is made of artificial material therefore there is a need to improve haemocompatibility. Further in vivo experiments are needed with a larger sample size to validate the results in this thesis.

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APPENDIX A

Gas Transfer Calculations

1.1 Equation for calculating the carbon dioxide content in blood

$$ctCO_2\,(B) = 9.286\times10^{-3}\times pCO_2\times ctHb\times\left(1+10^{\boldsymbol{pH_{ery}-pK_{ery}}}\right)+ctCO_2\,(\boldsymbol{P})\times\left(1-ctHb21.0\right)$$

$$pH_{\boldsymbol{ery}}=7.19+0.77\times(pH-7.40)+0.035\times(1-sO_2)$$

$$pK_{\boldsymbol{ery}}=6.125-\log\left(1+10^{\boldsymbol{pH_{ery}-7.84-0.06\times sO_2}}\right)$$

$$ctCO_2\,(P)=0.23\times pCO_2+cHCO_3\,(\boldsymbol{P})$$

$$cHCO_3\,(P)=0.23\times pCO_2\times10^{\boldsymbol{pH-pK\,(P)}}$$

$$pK\,(P)=6.125-log(1+10^{\boldsymbol{pH-8.7}})$$

Total concentration of CO_2 in whole blood: $ctCO_2\,(B)$ [mmol CO_2/L blood]; partial pressure of CO_2: pCO_2 [kPa], total concentration of hemoglobin: $ctHb$ [mmol Hb/L blood]; pH of erythrocyte (red blood cell): pH_{ery} [unitless]; pK of erythrocyte (red blood cell): pK_{ery} [unitless]; total concentration of CO_2 in plasma: $ctCO_2\,(P)$ [mmol CO_2/L blood]; concentration of bicarbonate in plasma: $cHCO_3\,(P)$ [mmol HCO_3^-/L blood]; pK of plasma: $pK(P)$ [unitless][1, 2].

1.2 Equation for calculating the oxygen content in blood

$$ctO_2\,(B)=0.000031\times pO_2+1.39\times sO_2\times ctHb_{(g)}$$

Total concentration of O_2 in whole blood: $ctO_2\,(B)$ [mL O_2/mL blood]; partial pressure of O_2: pO_2 [mmHg]; concentrational solubility coefficient for O_2 in plasma: 0.000031 [mL O_2 /mmHg/mL blood]; O_2 saturation: sO_2 [unitless]; total concentration of hemoglobin: $ctHb_{(g)}$ [g Hb/mL blood]; Huefner's constant (theoretical oxygen-carrying capacity of haemoglobin): 1.39 [mL O_2/g Hb][1-3].

(Rochow et al., 2013)

APPENDIX B

Micro Vascular Network Fabrication

1. A master of the micro vascular network was prepared using soft lithography.
2. Preheat oven to 80C.
3. Mix PDMS base and curing agent in a 10:1 Total volume of PDMS is dependent on number of SOU bodies required i.e. 9=8mL/ 1SOU body.
4. Stir mixture well with a stirring rod.
5. Place under vacuum for 10-15 minutes.
6. Place master on a levelled surface.
7. Cut two 8mm silicon tubing per each SOU body.
8. Place both tubing's at inlet and outlet position SOU master.
9. Pour 8mL of PDMS mixture onto master.
10. Place master under vacuum for 10-15 minutes.
11. Remove from vacuum and place in 80C oven for 1 hour.
12. Remove and cut out the body.
13. Remove residual PDMS from the inlet and outlet tubing's of the SOU body.
14. Place body in clean petri dish.

APPENDIX C

Fabricating Polycarbonate SOU

1. Prepare PDMS base and curing agent mixture in 10:1 ratio.
2. Obtain a silicon wafer and center onto spinner.
3. Pour 1mm of PDMS mixture in center of wafer.
4. Spin at 500 rev/ minute for 10 s and increase to 4000rev/minute for 30 s.
5. Remove wafer from spinner.
6. Spin one silicon wafer per SOU.
7. Place SOU body in low pressure oxygen plasma machine and treat (60W; 30sec).
8. Remove treated SOU body and stamp onto spunned silicon wafer with thin film of PDMS.
9. Leave for 10 seconds.
10. Remove body from the silicon wafer and bind to PC membrane.
1. Remove any wrinkles formed and lightly tap the area where channels are found.
2. Tap around the channels to ensure no air bubbles present.
3. Using a syringe with needle, draw up 1.5 mL of PDMS mixture and pour it around the oxygenator and membrane to ensure it is sealed.
4. Place in 80C oven for 1 hour.
5. Remove and store in clean petri dishes.

APPENDIX D

Fabricating PDMS SOU

1. Prepare PDMS base and curing agent mixture in 10:1 ratio.
2. Obtain wax film paper, and cut into circle with approximately 12 cm diameter.
3. Centre the wax film paper on spinner, and pour 1 mL of PDMS into the center.
4. Spin at 500 rev/ min for 10 s and increase to 4000rev/minute for 30 s.
5. Remove from spinner and place into petri dish.
6. Leave to cure overnight under ambient air.
7. Turn spinner on.
8. Prepare PDMS base and curing agent mixture in 10:1 ratio.
9. Obtain a silicon wafer and center onto spinner.
10. Pour 1mm of PDMS mixture in center of wafer.
11. Spin at 500 rev/ minute for 10 s and increase to 4000rev/minute for 30 s.
12. Remove wafer from spinner.
13. Spin one silicon wafer per SOU.
14. Place SOU body in low pressure oxygen plasma machine and treat (60W; 30s).
15. Remove treated SOU body and stamp onto spunned silicon wafer with thin film of PDMS.
16. Leave for 10 s.
17. Remove body from the silicon wafer and bind to PDMS membrane.
18. Leave overnight to cure under ambient air.

19. Once cured overnight, using a syringe with needle, draw up 1.5 mL of PDMS mixture (10:1 ratio) and pour it around the oxygenator and membrane to ensure it is sealed.
20. Place in 40C oven for 4-5 hours.
21. Gently remove wax film and paper from the PDMS oxygenator. The oxygenator must be completed cured, in order to ensure no damage occurs to the membrane.
22. Once wax film and paper has been removed, place in 80C oven for 1 hour. This will ensure the PDMS membrane is hardened.
23. Store PDMS oxygenator in petri dish.

APPENDIX E

Fabricating Porous PDMS SOU

1. Prepare PDMS base and curing agent mixture in 10:1 ratio. Add grounded 5%NaCl to the PDMS mixture. Stir rigorously to ensure even distribution of salt and PDMS mixture.
2. Obtain wax film paper, and cut into circle with approximately 12 cm diameter.
3. Centre the wax film paper on spinner, and pour 1 mL of PDMS into the center.
4. Spin at 500 rev/ minute for 10 s and increase to 4000rev/minute for 30 s.
5. Remove from spinner and place into petri dish.
6. Leave to cure overnight under ambient air.
7. Prepare PDMS base and curing agent mixture in 10:1 ratio.
8. Obtain a silicon wafer and center onto spinner.
9. Pour 1mm of PDMS mixture in center of wafer.
10. Spin at 500 rev/ min for 10 s and increase to 4000rev/minute for 30 s.
11. Remove wafer from spinner.
12. Place SOU body in low pressure oxygen plasma machine and treat (60W; 30sec).
13. Remove treated SOU body and stamp onto spunned silicon wafer with thin film of PDMS.
14. Leave for 10 s.
15. Remove body from the silicon wafer and bind to porous PDMS membrane.
16. Leave overnight to cure under ambient air.

17. Once cured overnight, using a syringe with needle, draw up 1.5 mL of PDMS mixture (10:1 ratio) and pour it around the oxygenator and membrane to ensure it is sealed.
18. Place in 40C oven for 4-5 hours.
19. Gently remove wax film and paper from the PDMS oxygenator. The oxygenator must be completed cured, in order to ensure no damage occurs to the membrane.
20. Once wax film and paper has been removed, place in 80C oven for 1 hour. This will ensure the PDMS membrane is hardened.
21. Prepare a 80C hot water bath.
22. Gently put porous oxygenators into hot water bath and leave for 4 hours.
23. Subsequently, remove the oxygenator from the hot water bath, and store in petri dish to dry.