

Fabrication of Oxygenation Microfluidic Devices for Cell Cultures

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Abstract

Current cell culture procedures that use normoxic (21%) oxygen concentrations have been proven to be physiologically inaccurate since the oxidation conditions in the human body ranges mostly between 1-13%. Recent studies have acknowledged this fact and have begun utilizing microfluidic devices to create an oxygen gradient to accurately depict the human physiology. While these studies have taken a step in the right direction, the gradient achieved is rather steep posing a spatial constraint in accurate testing. In order to address this problem we have fabricated microfluidic devices of five different specialized micro-channel architectures and tested them to find the optimal low rise oxygen gradient. Through fabrication and testing of multiple batches, we have determined that one of our specialized micro-channel designs (Architecture 3) has successfully produced a low rise oxygen gradient that provides ample spatial resolution to accurately pinpoint oxygen concentrations of interest, paving the way to improve the accuracy of cell culture techniques.

1. Introduction

The current standard of cell culture includes incubation and sterile hood techniques that promote cell growth *in vitro*. It however ignores the fact that the oxygen concentration levels inside a human body is much lower than the atmosphere varying from 1% in the cartilage to 7% in the bone marrow to 10-13% in the arteries, lungs, and liver.² The cells that have been cultured under standard techniques thus might not behave the same way they do under their natural oxygen concentrations.⁴ Recent studies have acknowledged this fact and more research using microfluidic devices have been done in order to mitigate this problem.³ The high gas permeability of polydimethylsiloxane (PDMS) has been utilized to fabricate specialized micro-channel network contained within a microfluidic device. Multiple sources of oxygen concentration were used as input to form an oxygen gradient within the specialized micro-

channel network.¹ These microfluidic devices with its micro-channel networks have already improved upon standard oxygen-based culturing methods.³

While these studies have taken a step in the right direction, further studies must be done to optimize the oxygen gradient produced through these microfluidic devices. These studies have successfully used microfluidic devices to produce an oxygen gradient; however these gradients have been very steep. The size of the microfluidic devices used has ranged from 1mm – 8mm.^{1,3} This steep gradient alongside the small length of the device poses a spatial constraint that makes accurate determination of oxygen concentrations difficult. A larger device with a low rise gradient would provide ample spatial conditions to accurately determine oxygen concentrations of interest. In the following studies, various micro-channel architectures of varying pillar size and separation were designed using AutoCAD. The length of the device was set to 57mm for all the architecture designs. The micro-channel architecture designs were translated into specialized micro-channel network through a photomask carrying these designs, using photolithography followed by soft lithography. The micro-channels along with its other components were fabricated and bonded to assemble the microfluidic device. The device was then tested using an oxygen probe setup that measured oxygen concentration along discrete intervals across the device. Multiple identical devices were fabricated and tested for each micro-channel architecture design and the oxygen gradients produced were compared to other micro-channel architecture designs. A total of four varying micro-channel architecture designs were tested along with an alternative design.

2. Methods

2.1 PDMS microfluidic device fabrication

A silicone elastomer commonly used in soft lithography namely Polydimethylsiloxane (PDMS) was used to fabricate the microfluidic devices. Its optical transparency, low toxicity and high coefficient for oxygen diffusion made it an ideal choice.⁵ A PDMS pre-polymer (Sylgard 184, DOW) was mixed with its curing agent maintaining a 10:1 ratio. The air bubbles trapped inside the substrate was then removed using a vacuum chamber. The PDMS was then used to fabricate various components of the device; the microfluidic channels, gas permeable membrane and chambers to form a well.

Various photomasks with varying micro-channel architecture were designed using AutoCAD. The photomasks were then used to design masters of corresponding architecture using photolithography. SU8, a negative photoresist was spun into a plasma treated silicon wafer four inch in diameter. The spin speeds were set to 500rpm for 10 seconds followed by 1400rpm for 30 seconds to achieve a desired thickness of 200 μ m. The wafers were then baked on a hotplate at 65°C for 6 minutes followed by 95°C for 39 minutes. The photomasks were then used to selectively expose UV radiation to desired locations on the wafer. The areas in the wafer corresponding to the transparent portions of the photomasks underwent cross-linking as they were exposed to UV radiation for a set amount of time. The wafers were then baked again at

65°C for 5 minutes and 95°C for 14 minutes. The wafers were then placed on a shaker in a solution of SU-8 developer for 16 minutes followed by 10 minutes in a new SU-8 developer. The portions that were not cross-linked were dissolved by the developer leaving a mold of SU-8 architecture (master). After silanization in a vacuum chamber, the masters were then used as a platform to fabricate PDMS microfluidic channels using soft lithography. PDMS was poured onto the master bounded on all sides using micrometer glass slides and covered by a transparency layer. A weight was used to provide pressure on top and the wafer was baked at 65°C for 90 minutes. The PDMS microfluidic channels were then extracted and stored.

PDMS folds were fabricated by pouring 50 grams of PDMS in a container and baking it at 65°C for 120 minutes. The PDMS mold was then cut to form wells. The gas permeable membrane was fabricated by spin coating PDMS in a wafer 4 inch in diameter. The spin speeds were set to 500 rpm for 10 seconds followed by 800 rpm for 30 seconds to achieve the desired thickness of 100 μ m. The three components namely the well, gas permeable membrane and the microfluidic channels in respective order from the top were bonded using surface plasma treatment. A hole of varying size and position corresponding to the architecture were punctured to form the inlets and outlets. A glass slide was then used as base to plug the inlets/outlets and provide mechanical support for the device. The glass slide along with the other components was then plasma treated and baked at 65°C for 120 minutes to form the fully integrated microfluidic device.⁵

2.2 Alternative device architecture

A master which used only one pair of inlets/outlets was used to form an alternative architecture. The device used similar methods of photolithography followed by soft lithography to fabricate and assemble devices except of one major difference. In the other microfluidic devices multiple pairs of inlet/outlet were used with varying oxygen concentration in each inlet to create the oxygen gradient. The gas permeable membrane was of constant 100 μ m thickness. In the alternative architecture, one oxygen concentration was pumped through one solitary pair of inlet/outlet while a membrane of varying thickness (inclined membrane) was used to achieve the oxygen gradient. Scotch tapes stacked on top of each other were used to fabricate the inclined membrane. Four pairs of Scotch tapes with varying thickness were distributed uniformly across a silicon wafer, 4 inch in diameter. The thickness of Scotch tapes was measured to be 100 μ m, 200 μ m, 300 μ m and 400 μ m respectively across the wafer. PDMS was then poured onto the wafer and covered with a transparency layer. A weight was used on top to provide pressure and the wafer was baked at 65°C for 60 minutes

3. Results

3.1 Device Setup

The microfluidic device was connected to multiple gas tanks depending on the number of inlet/outlet pairs the architecture had. The micro-channel architecture in our microfluidic devices ranged from having one solitary pair to having as many as 7 pairs of inlets/outlets. The oxygen

probe was calibrated for hypoxic oxygen concentration using Sodium sulfite as an oxygen scavenger and calibrated for normoxic oxygen concentration using ambient conditions. The well of the device was filled with water and the tip of the oxygen probe was dipped into the water to take measurements.

3.2 Data Collection

The oxygen probe started from one end of the device and moved at regular intervals across the length of the well. The number of intervals varied as per the architecture designs and ranged from 7 to 15 data points distributed regularly across the length of the well. The oxygen probe was allowed to settle for 20 minutes before a measurement was taken at each data point. Two replicate measurements were taken at each datapoint for each individual device. Three identical devices were prepared for each micro-channel architecture designs leading to six replicate measurements along each data point for each individual architecture. The error bars were calculated using the standard deviation of these six replicate measurements across each data point.

3.3 Oxygen Gradients

4. Discussion

4.1 Device functionality

The microfluidic devices were arbitrarily chosen to be tested for device functionality. We found that around 20-30% of the devices we made failed to bond properly. Humidity has been known to cause similar problems. Furthermore, some of the devices were diagnosed by pumping a red dyed PDMS fluid into the micro channels using a syringe pump. The red fluid spread across the microfluidic channel revealing any clogged portions where the membranes may have collapsed onto the channel. The diagnostic is however a conservative one considering that the red dyed liquid is much heavier than oxygen and thus portions clogged for the liquid might still be permeable for oxygen.

4.2 Analysis and Future work

Among the five micro-channel architecture designs we tested, we found architecture 3 to have produced the best oxygen gradient. The oxygen gradient produced through architecture 3 design is fairly consistent and reliable. The replicate measurements are fairly precise as evident from the reasonable size of the error bars. The gradient maintains a good trend and provides ample spatial conditions for accurate testing of substrates. The zero error present in most of our measurements is due the fact that the wells lie some distance away from the inlets. There is a slight bump in the gradient at 40-50mm data point which we believe to be due to the non-uniform rise of oxygen concentration input (from 10% to 21%). An automated oxygen delivery system that can input accurate oxygen concentrations could be useful in solving this problem. An important issue to note was that there were some issues with PDMS-glass bonding while

fabricating some of the devices. Strict regulation of humidity could be implemented in order to mitigate these problems.

5. Conclusion

Through production and testing of multiple batches of microfluidic devices with varying micro channel architectures, we can safely conclude that the micro-channel architecture designs used, particularly architecture 3 performs their intended functionality and produces a reliable low-rise oxygen gradient. The larger size of the wells used in these microfluidic devices also afford a spatial resolute gradient of oxygen concentrations. While further testing must be done, especially in terms of using COMSOL simulations to determine further characteristics of the micro-channel architecture and biological testing with actual cells; the findings of this study can be useful in improving cell culture techniques everywhere. The results show a low rise oxygen gradient that provides ample spatial resolution to accurately pinpoint oxygen concentrations of interest, paving the way to improve the accuracy of mimicking human physiology better.

6. Acknowledgements

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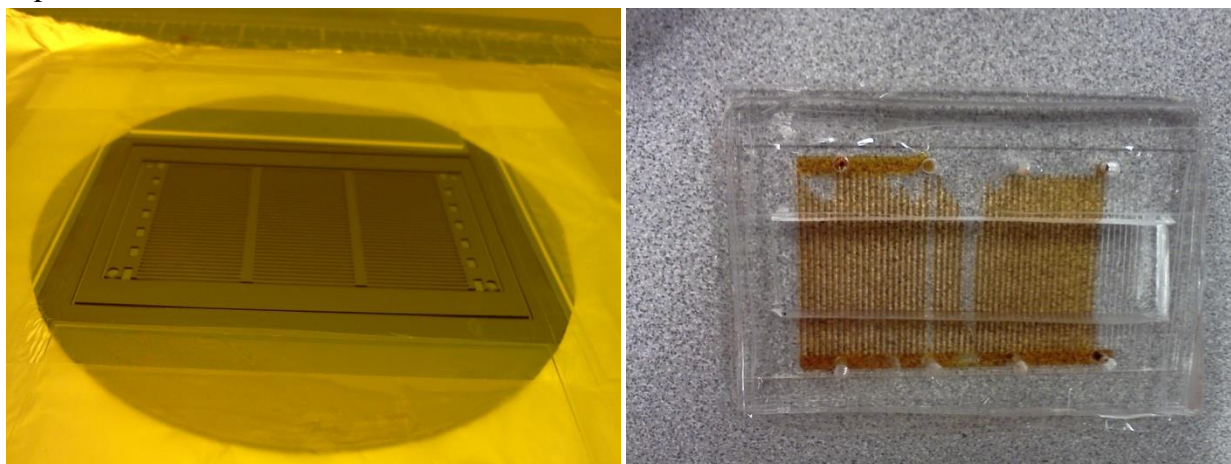


Fig 1: (left) A master mold designed using photolithography, being prepared for soft lithography. (right) A fully integrated microfluidic device partially dyed to visualize the micro-channel architecture.

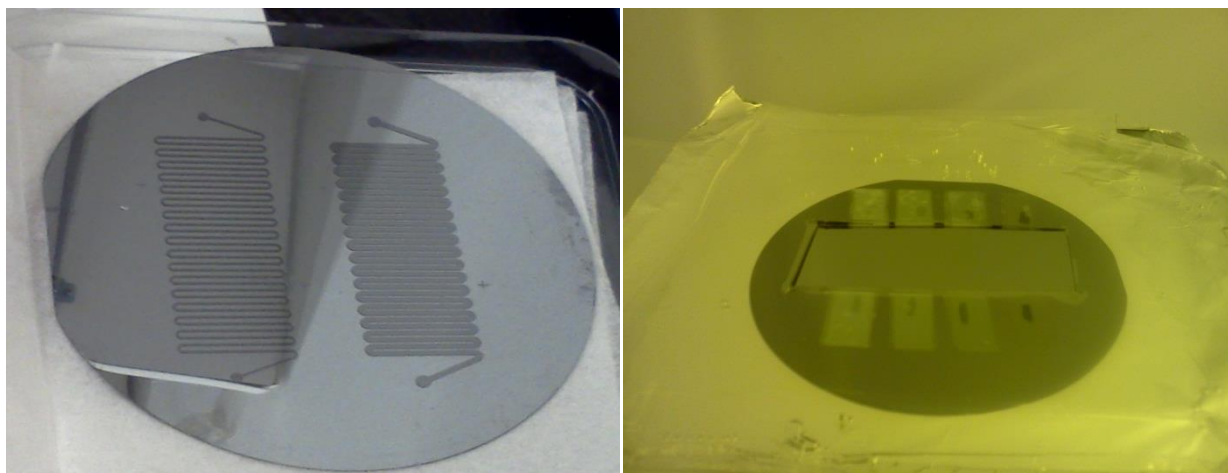


Fig 2: (left) A master designed using one solitary pair of inlet/outlet for the alternative device module. (right) The master mold with the inclined membrane taken out

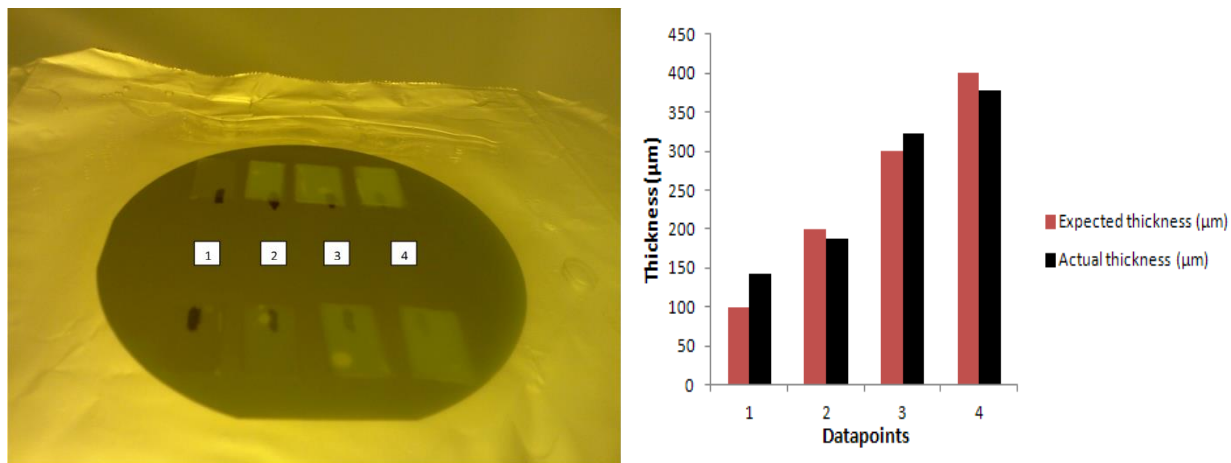


Fig: 3: (left) The arrangement of Scotch tape stacks with thickness of 100 μm , 200 μm , 300 μm and 400 μm respectively from the left. (right) Thickness measurements of the inclined membrane compared to the thickness of the Scotch tape used across the respective data points



Fig 5: The direction of the oxygen probe for each individual device.

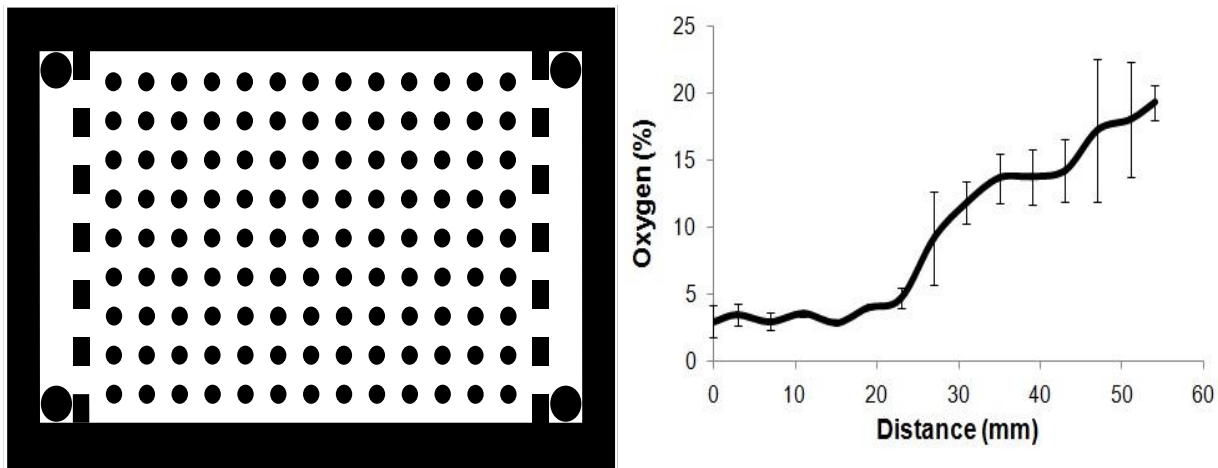


Fig 6: (left) Micro-channel Architecture Design 1. Two pairs of inlets/outlets; one inlet at 0% oxygen and the other at 21% oxygen. (right) Oxygen gradient observed using Architecture 1

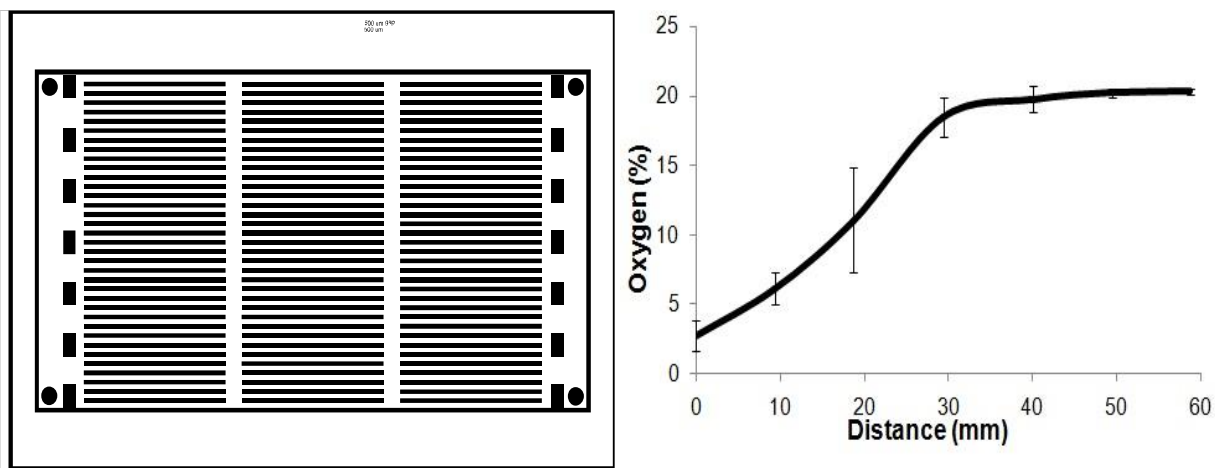


Fig 7: (left) Micro-channel Architecture Design 2. Two pairs of inlets/outlets; one inlet at 0% oxygen and the other at 21% oxygen. (right) Oxygen gradient observed using Architecture 2

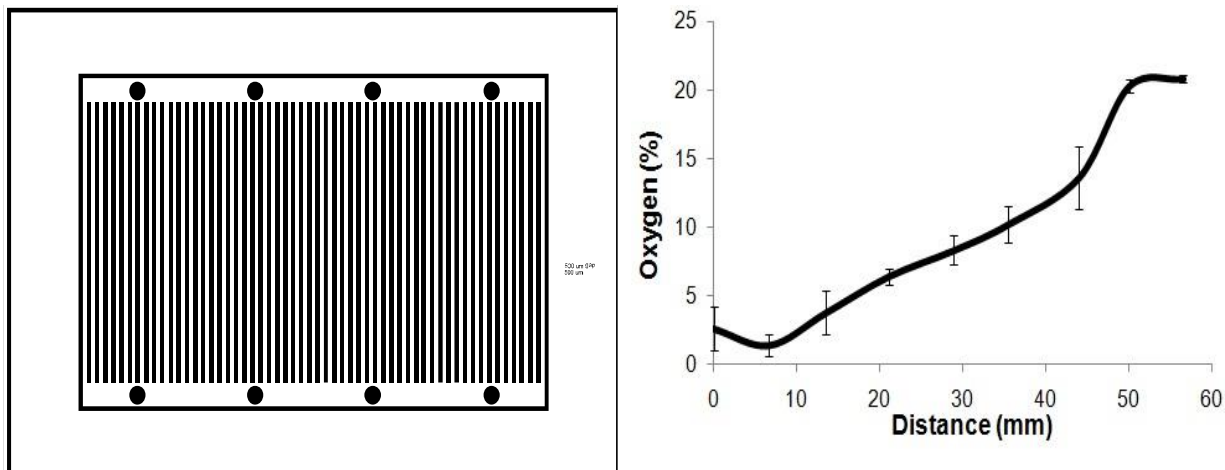


Fig 8: (left) Micro-channel Architecture Design 3. Four pairs of inlets/outlets; one inlet at 0% oxygen, next one at 5% oxygen, next one at 10% oxygen and the last one at 21% oxygen. (right) Oxygen gradient observed using Architecture 3

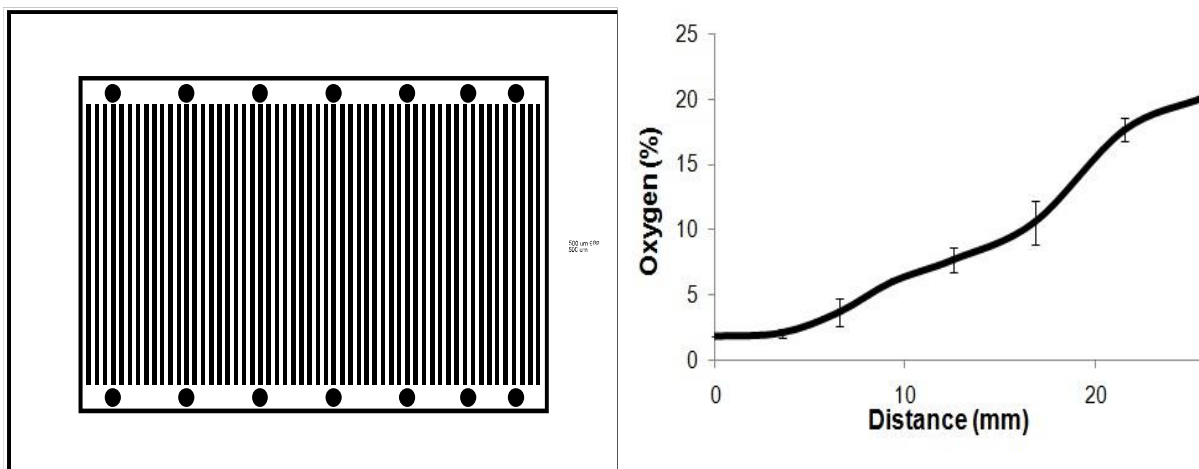


Fig 9: (left) Micro-channel Architecture Design 3. Seven pairs of inlets/outlets. Only four pairs starting from one end of the device were used however due to lack of enough oxygen concentration inputs. Only half region of length of the well (~28.5mm) tested. [starting from the left end] one inlet at 0% oxygen, next one at 5% oxygen, next one at 10% oxygen and the last one at 21% oxygen. (right) Oxygen gradient observed using Architecture 4

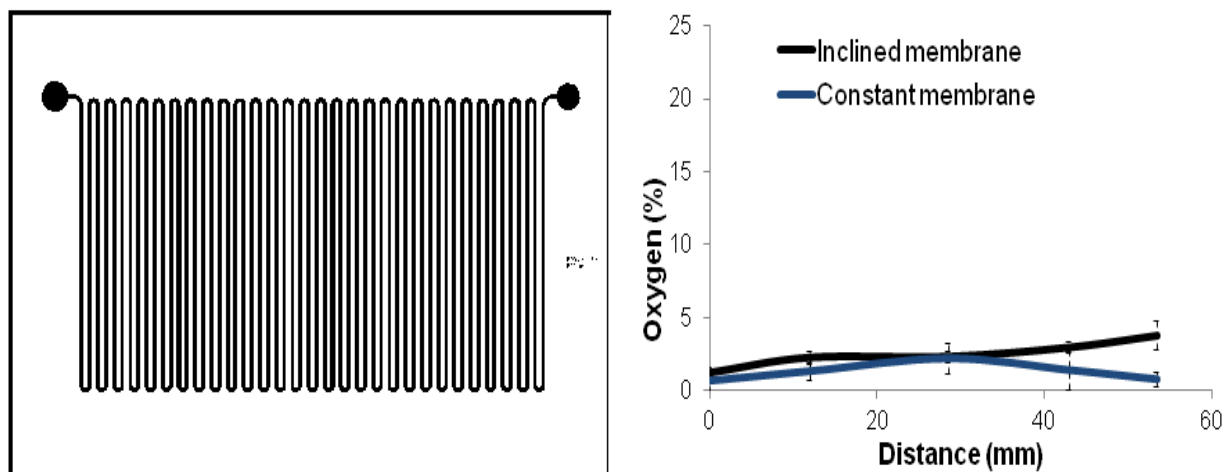


Fig 10: (left) Alternative Micro-channel Architecture Design. One solitary pairs of inlet/outlet; inlet at 21% oxygen. (right) Black line represents oxygen gradient observed using Alternative Architecture and an inclined membrane. Blue line represents oxygen gradient observed using Alternative Architecture but with the same traditional membrane of constant thickness; used as a control

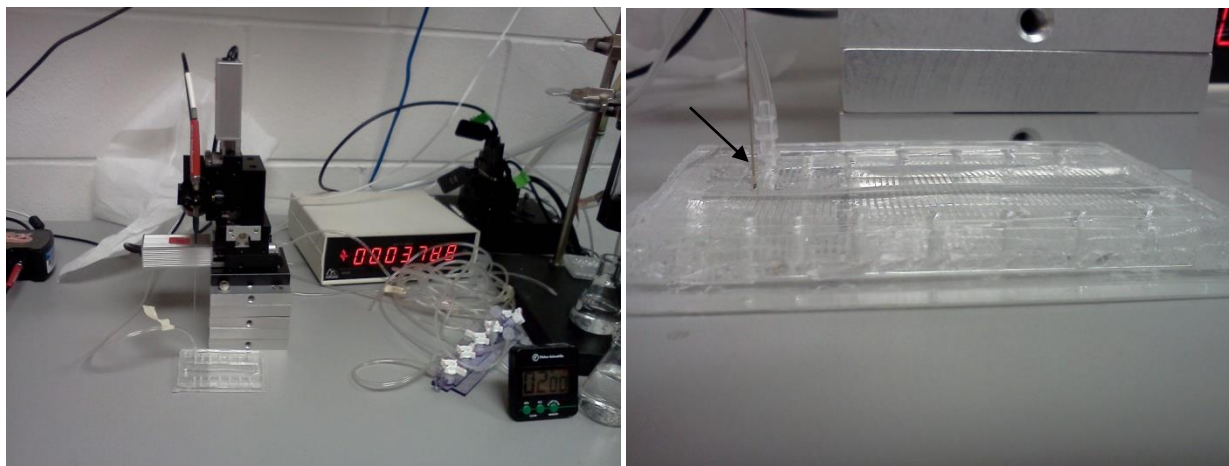


Fig 4: (left) Device setup using one input gas concentration. (right) Oxygen probe dipped into the well to take measurements

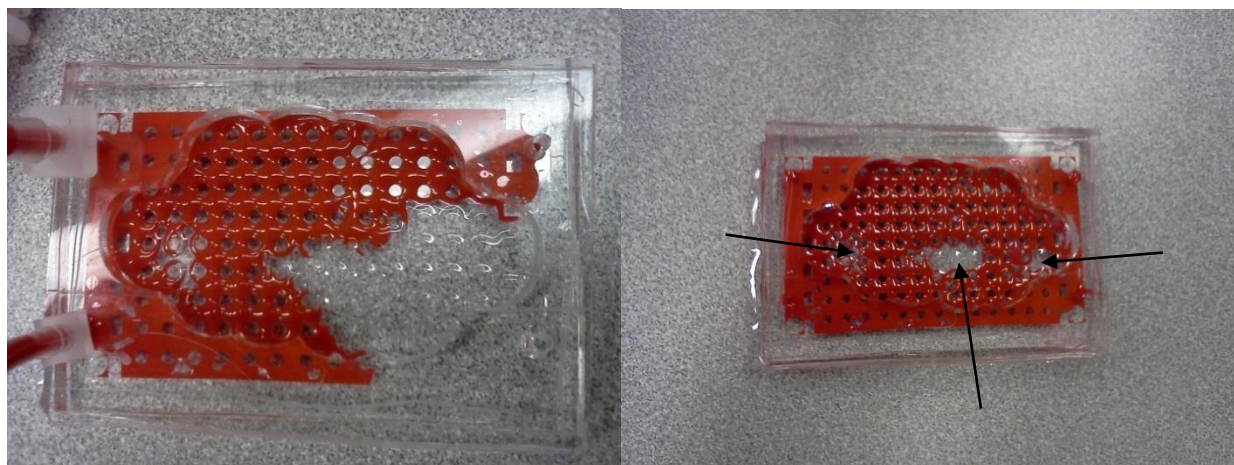


Fig 11: (left) A syringe pump being used to pump red dyed PDMS substrate into microfluidic channels. (right) The microfluidic channels when the pumping is completed. Clogged portions can be seen within the device