Microfluidic Chip Development and Testing

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Abstract

The placenta represents one of biology's most important membrane's, yet the study of the characteristics of said membrane is very difficult to simulate in the lab. While there have been methods of creating microfluidic chips to test the biomechanics of this membrane, they require complicated and expensive manufacturing processes. The microfluidic device described in this paper was created with the intent of testing the biomechanic characteristics of the amnion membrane with the use of materials and methods more commonly found in laboratories. The results show that although further testing is required, the microfluidic chip was successful in terms of creating a flow that could be used to test the characteristics of the amnion membrane ex-vivo.

Introduction

For humans as well as other mammals, the placenta plays a vital role in successful reproduction. Its most important function is the exchange of endogenous and exogenous substances, which enables adequate supply of oxygen and nutrients, excretion of fetal metabolic waste, and protection against potentially harmful agents such as xenobiotics, bacteria, viruses, and parasites [1-3]. The transfer of material between the intervillous space and fetal capillaries takes place across a multilayered "placental barrier" [1,3]. To be able to study this barrier usually requires the use of in vivo models or animal testing to truly have a model which is similar to the human placenta.

The placenta represents one of biology's most important membrane's, yet the study of the characteristics of said membrane is very difficult to simulate in the lab.

While there have been methods of creating microfluidic chips to test the biomechanics of this membrane, they require complicated and expensive manufacturing processes which use similar methods to that of created silicon wafer chips [4]. The microfluidic device described in this paper was created with the intent of testing the biomechanic characteristics of the amnion membrane with the use of materials and methods more commonly found in laboratories. While previous paper “Microfluidic Chip Development and Testing” focused more on development and design of the chip, this paper is aimed towards the testing and results acquired from the first prototype of the microfluidic device.

The goal of the microfluidic chip being described in this paper is to simulate as closely as possible the placental membrane in humans to be able to test the membrane’s biomechanics such as mass transfer characteristics. To do so a microfluidic chip with isolated input and output channels was created to closely represent the microfluidic chip described in the “Placenta-On-A-Chip” paper[1]. The secondary goal was to create this device using simpler methods, with equipment more commonly found in labs, and with a more time efficient process. Currently the microfluidic chips are mostly developed using a photolithography technique similar to designing printed circuit boards. This method is time consuming and many times very expensive, especially considering that for testing many of these must be produced. To create a cheaper microfluidic chip with simpler manufacturing processes would enable cheaper and more available methods of ex-vivo testing of the amnion membrane.

The main purpose of this study is to determine the transfer characteristics of different molecules, such as glucose, NaCl, urea, and many other molecules that play

an important role in the everyday functions of cells. To do so, a method and device for testing the chip would also have to be developed to be able to reliably test the microfluidic chips, which would ultimately help simulate in vivo process such as molecular transport. Mimicking molecular transport across the placenta membrane would allow us and other researchers to study the affects of different drugs, nutrients, or other molecules, specifically relating to embryonic development. Creating this improved experimental setup became a major part of the project once the limitations of the first test setup were realized. The authors of the “Placenta On a Chip” paper did not provide, in their methods and materials, the exact equipment and experimental setup of the equipment, which meant that new methods and equipment were developed in an attempt to produce a similar testing environment.

Testing of the chip was done so using methods of concentration change described in the article “Placenta-on-a-chip: a novel platform to study the biology of the human placenta”. By doing so, any given substance could be used and transfer characteristics could be determined for said substance across the amnion membrane. Testing substances and how they interact with the amnion membrane could provide in-depth insight into what pregnant women should and should not be consuming, such as types of food, pharmaceuticals, and possibly other substances that may enter the bloodstream and interact with this membrane.

Materials and Methods

To be able to test the biomechanics of the amnion membrane, the microfluidic chip needed to be developed using techniques and materials available in the lab. The

materials and methods used were aimed towards being able to manufacture this microchip consistently, in a short time frame, and using less complicated methods than mentioned in “The Placenta-On-A-Chip” paper [1]. The materials used in the construction of the chip were 250 micron thick Polydimethylsiloxane (PDMS) sheets, silicone tape, and continuous cast acrylic sheets. The specific PDMS sheets, SSP-M823, were purchased from a company, SSP, which provides various different thicknesses for the PDMS sheets, ranging from .005” to 0.040”. The specifications of these PDMS sheets can be found on their website. These raw materials are then laser cut accordingly and assembled around the amnion membrane to be tested. The Universal Laser Systems’ 70 Watt laser cutter along with compatible software would be the primary tool being used to develop the chip due to its precision cutting, necessary to cut the 500 micron width and 250 micron height in the channels. Corel was the CAD software used to create a template for the laser cutter which would cut the distinct layers of PDMS to be assembled later. The CAD template to be cut can be seen in figure 1 and an exploded view of the different, specific layers can be seen in figure 3. To produce the channels of with the appropriate dimensions, several layers of PDMS, with specific patterns, were adhered to each other using silicone tape. The PDMS, being 250 microns thick, provided the adequate height for the channels. Therefore, by cutting completely through this 250 micron thick material in the specified patterns, which can be seen in figure 1, we were able to create the desired channel height of 250 microns. The width of the channels was created by the laser cutter, by cutting out 2D channels of 500 microns, in width, in the specified layers of PDMS. This would ultimately create the

channels with a width and height of 500 x 250 microns, respectively. Once the microchip was cut to specifications and assembled, efficiency testing could be conducted.

Laser Cutter and Settings Used

Since the laser cutter, would play a large part in the manufacturing of this microfluidic chip it was necessary to calibrate the settings to produce the most precise cuts in each of the three of the materials used. For the 1.5 mm thick acrylic sheet the best cuts occurred when the setting were power=100, speed=80, PPI=708, and Z-axis=1.5mm. These settings were used and the laser cutter repeated this pattern ten times to ensure the laser had cut entirely through the material. This acrylic layer is used as structural support in the chip as well as an interface for the input and output tubes to attach. The settings for cutting the silicone tape and the PDMS were the same as for the acrylic except for the Z-axis was set to 0. For cutting the silicone and PDMS the external nitrogen gas was turned on which helps instantly cool the material once the laser cuts it, making for a less distorted and more precise cut. Also, only 4 repetitions of the cut pattern were necessary to cut completely through each the silicone tape and PDMS materials. The repetition number of cuts is set in the Corel CAD software by stacking identical pattern layers on top of each other. The cut patterns can be seen in Figure 1.

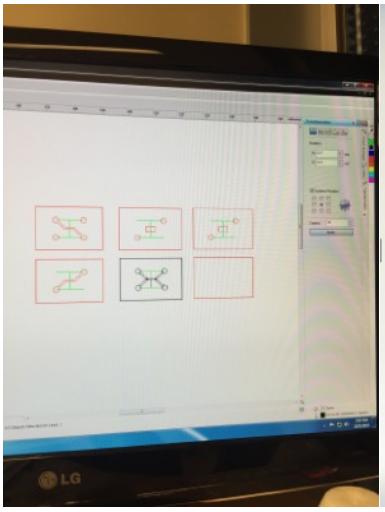

Figure 1
Corel Drawing

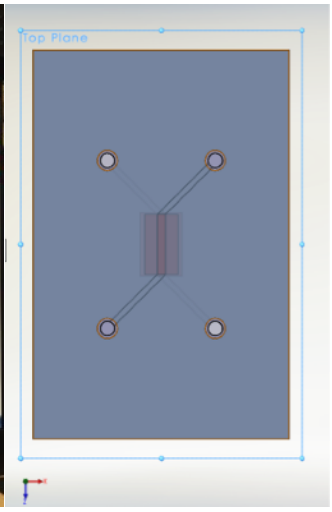

Figure 2
Solidworks CAD Rendering

Manufacturing Process

The build started with a CAD rendering similar to the one seen in Figure 2 which is based on the microfluidic chip developed by the authors of “Placenta-On-A-Chip”. The dimensions of Figure 2 are 15.3 mm (width) X 23.3 mm (height), which are also the outer dimensions for every layer in the chip minus the actual placenta membrane being used. The amnion membrane was cut to roughly .25cm X .38 cm using a scalpel. The final thickness of the finished chip is about 4.5 mm.

For the build process, large enough pieces of PDMS and silicone tape are placed into the laser cutter bed (note that the PDMS has two protective layers which should be removed prior to being placed in laser cutter). Layers are cut using the laser cutter according the Corel drawing then assembled according to the schematic shown in Figure 3. The clear layers represent PDMS, the blue layers silicone tape, and the green the acrylic supports. The silicone tape simply serves to create a watertight seal between the layers. The amnion membrane is cut to be slightly larger than the area of the channel in the adjacent, sandwiching silicone-tape layers to ensure that nothing will be allowed to flow around the membrane. This overlap of the placenta, shown in red, can be seen in Figure 4, which also gives a cross-sectional view of the input and output channels which

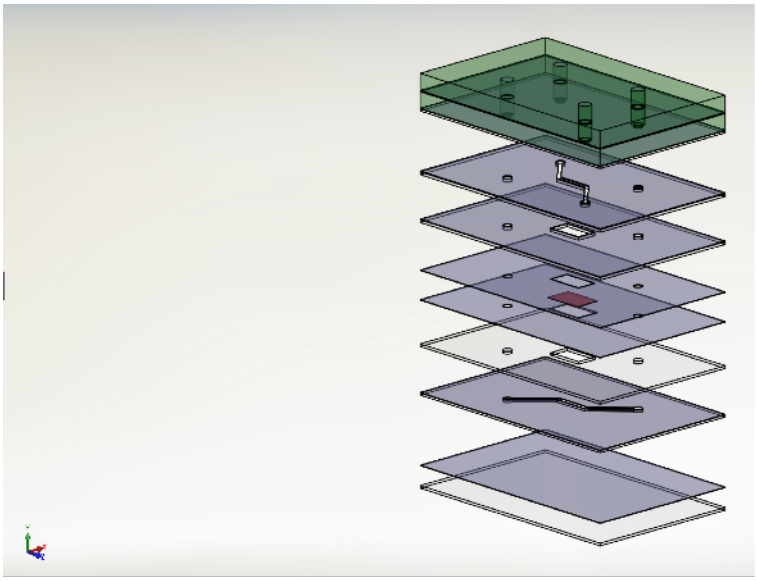

Figure 3
Exploded view, microfluidic chip assembly

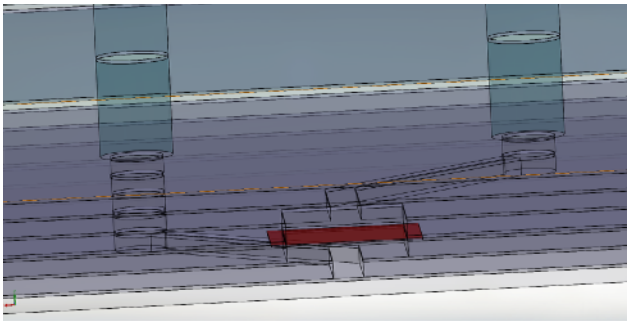

Figure 4
Cross sectional cut. Input/Output channels

are shown above and below the placenta. From Figure 4 it is easy to see how the device works. By having the left channel be the input the solution flows to the channel directly underneath the membrane and we can study how much of this solution flows through the membrane, to the channel above, to the output.

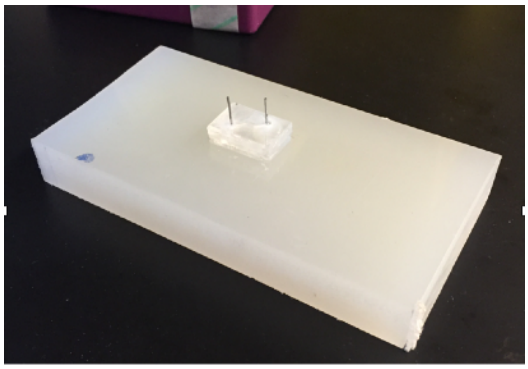

Figure 5
Alignment block

Assembly Methods

Once this method of manufacturing proved effective, a few steps and techniques were added to help in the ease of production as well as increase its reproducibility. Figure 5 depicts the alignment tool developed to ensure that the inputs, outputs, and channels were all perfectly aligned. The only limitation in this tool was that only the layers that contained the input/output channels could be assembled using this template while the solid end piece would have to be applied after by hand. Misalignment in the end piece would not result in the chip being ineffective which made this process acceptable. The next technique used to save time and help in the assembly of the chip was bonding the silicone tape to the PDMS sheet prior to being laser cut. This meant that assembling the layers was a matter of peeling the silicone tape's

Figure 6
Finished microfluidic chip with amnion membrane

protective sheet off and sticking the layers together with slight pressure being applied. The total time required to manufacture this microfluidic device is about 45 minutes to an hour. The finished chip with can be seen in Figure 6.

Verification Test

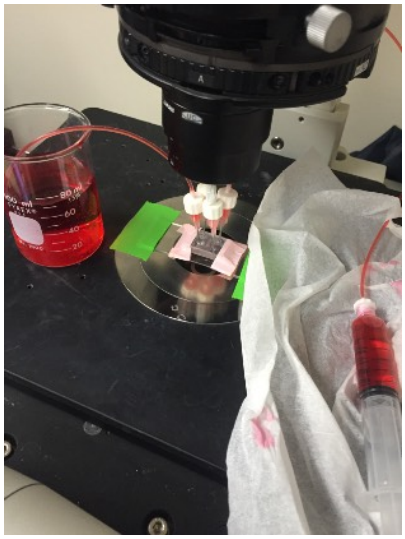

Figure 7
Verification testing of the microfluidic chip. One input consists of red dye and the other channel is filled with water

Before the transfer characteristics of different molecules could be tested across the placenta it was necessary to determine whether the construction of the microfluidic chip was successful and it was operating normally. To do so a microfluidic chip was constructed and connected to two input and output tubes. One channel input had red dye pumped into the channel using a 10mL syringe, which can be seen in figure 7. The other channel was filled with water, but had no input flow. The chip was observed under a microscope to visually determine if there were any leaks, which is why having two different colors was necessary. In figure 8 we see the up close picture of the different channels and the red dye, while difficult to see, is in only one of the channels.

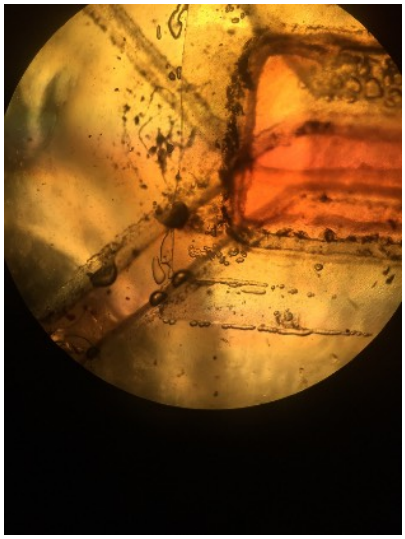

Figure 8
Close up microscope picture of verification testing

Test Setup Using Microfluidic Pumps

Two HARVARD apparatus (PHD 2000) microfluidic pumps were used as a controlled input to supply the two input tubes of the microfluidic chip with whatever solution was being tested. This provided a controlled method to conduct experimental

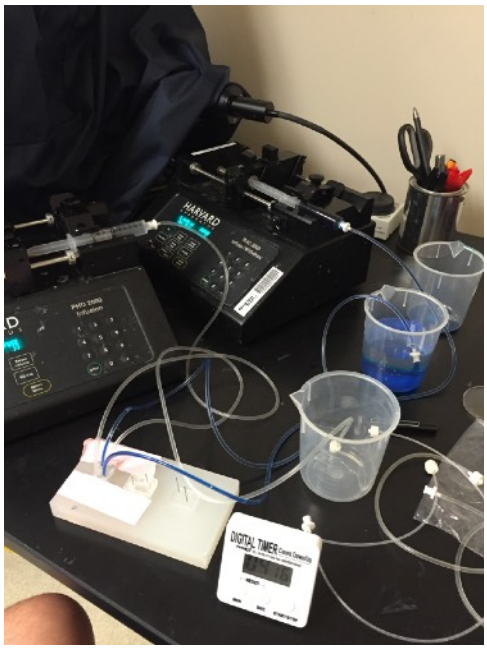

Figure 9
Test setup using HARVARD PHD2000 microfluidic pump to supply solutions to both inputs. In this case, one was blue dye and the other water

trials. Figure 9 shows this experimental setup. The major limitation to this setup was the duration of the experimental trials, which was about five minutes. For this reason, another method for conducting experimental trials was deemed necessary to allow for prolonged testing.

Testing Device For Continuous Trials

Although we will discuss the results of testing done with the Harvard microfluidic pumps later on, it is important to note that the development of this new testing device was primarily due to inadequacies in the first method of conducting experiments. The main advantage to this continuous microfluidic device seen in figure 10 is that it allows for autonomous tests that can run indefinitely while logging the data being measured. This is important considering the HARVARD pumps could only run for about 5 minutes before needing to be reset and the “Placenta On A Chip” paper ran their trials for 68 hours at a time and then compiled results. With this setup, we plan to continuously measure pH and conductivity of the output in both channels due to the first trials being done with NaCl solution, mainly because of its prevalence in the bloodstream and ease of measuring concentration. Due to components not having been delivered, the current device only has one pH meter to determine changes in a single output reservoir and conductivity is measured with a HM Digital TDS-3 conductivity meter by hand. This allows us to determine if a solution or molecule has transferred over to another channel, through the

Figure 10
Continuous Microfluidic device with the control interface connected to a laptop for debugging

membrane, by measuring the change in a certain measurement in a single output reservoir.

The continuous microfluidic device is powered by an Arduino micro controller which recycles the output back into the input using two water pumps. The device is comprised of four reservoirs, two inputs and two outputs, which are connected the microfluidic chip under test. The input reservoirs have water level sensors that react to low levels of solution and alert the pumps to recycle the output back into the input. This allows the device to run for an indefinite amount of time. The output reservoirs will be monitored by pH and conductivity sensors which log their measurements to an SD card connected to the Arduino at a specific time interval. This allows us to see the total change of pH or conductivity during the experimental trial, which we can relate to concentration by creating a standard curve.

Results

The following shows the data collected across many aspects of this study, including the verification testing, flow characteristics, and standard curves for solution concentrations.

Verification Testing

The verification testing done for the microfluidic chip consisted mainly of qualitative measurements to ensure the chip worked as it was supposed to. The following images, figures 11 and 12, depict how these subjective qualifications where measured.

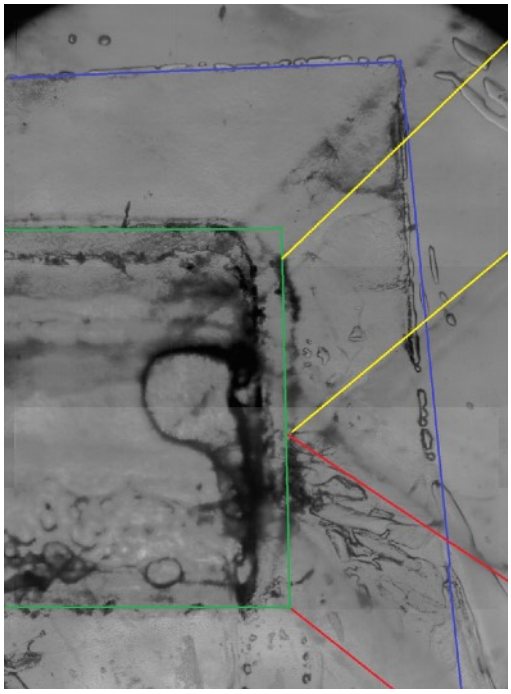

Figure 11
Black and white Microscope close up of microfluidic chip (Objective 4X). Yellow lines outline the upper channel, red lines outline the lower channel, the green box outlines the area of where transfer occurs, and the blue outlines the placenta membrane

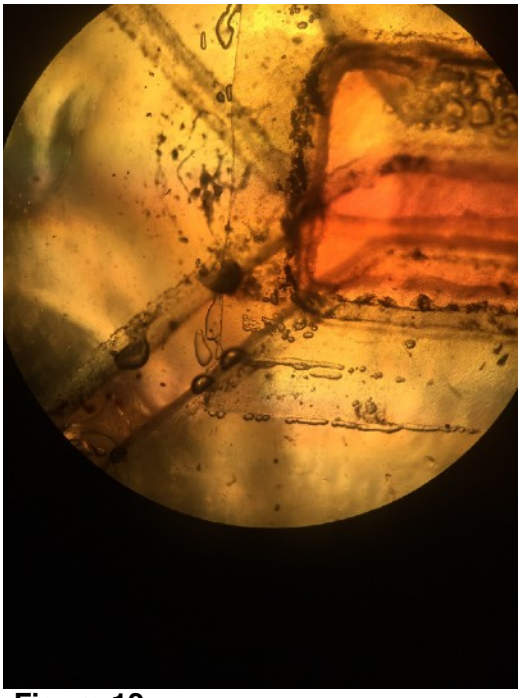

Figure 12
Color Microscope close up of microfluidic chip (Objective 4X) with red dye in upper channel.

Concentration Standard Curves

Concentration of Blue Dye in solution				
Number of Drops per 5ml of water	Absorbance (325nm)	Transmittance(325 nm)	Absorbance(460n m)	Transmittance(460 nm)
0	0	0	0	0
1	1.058	8.8	0.084	82.4
2	2.142	0.7	0.199	63.2
3	3.028	0.1	0.305	49.6
4	3.343	0	0.391	40.6
5	3.339	0	0.501	31.6

Table 1: Using a VIS/UV spectrophotometer to measure absorbance and transmittance of water with blue dye.

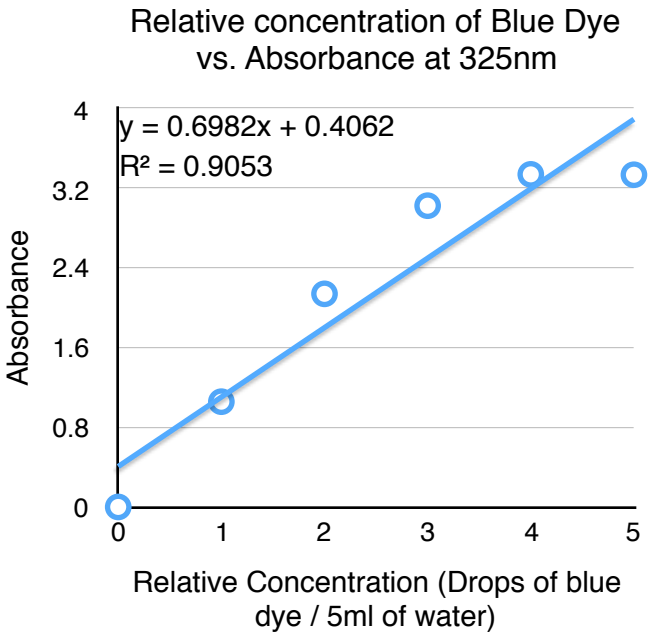

Figure 13: Standard curve for relative concentration of blue dye vs. absorbance at 325nm

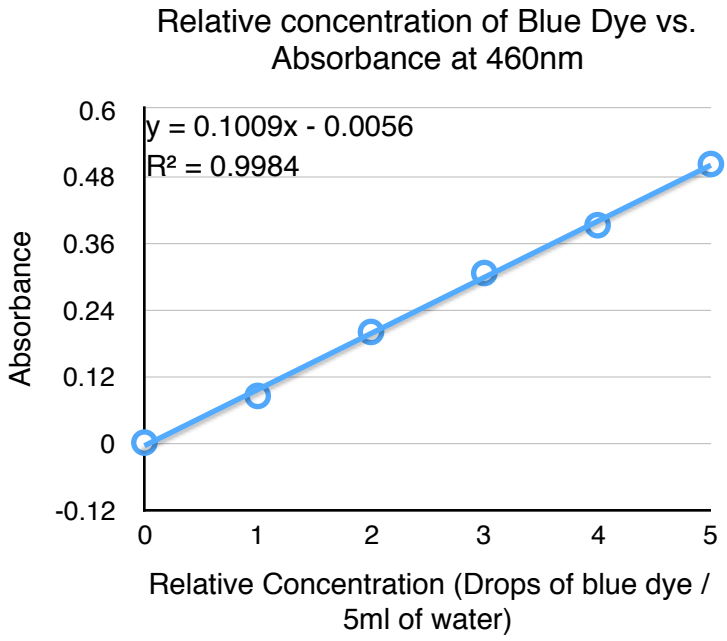

Figure 14: Standard curve for relative concentration of blue dye vs. absorbance at 460nm

Conductivity & pH vs Concentration of NaCl							
Molar Mass NaCl (g/mol)	mL Water	L Water	G NaCl	Moles of NaCl	M Solution (mol/L)	Conductivity (PPM)	pH
58.44	473.176	0.473176	0	0.00000	0.00000	71	7.32
58.44	473.176	0.473176	1.37	0.02344	0.04954	2630	7.09
58.44	473.176	0.473176	2.45	0.04192	0.08860	4980	6.98
58.44	473.176	0.473176	3.64	0.06229	0.13163	7150	6.85
58.44	473.176	0.473176	4.79	0.08196	0.17322	9950	6.79

Table 2: Conductivity and pH of differing concentrations of NaCl in water. pH measured with ATC pH meter.

Conductivity measured with HM Digital TDS-3 probe.

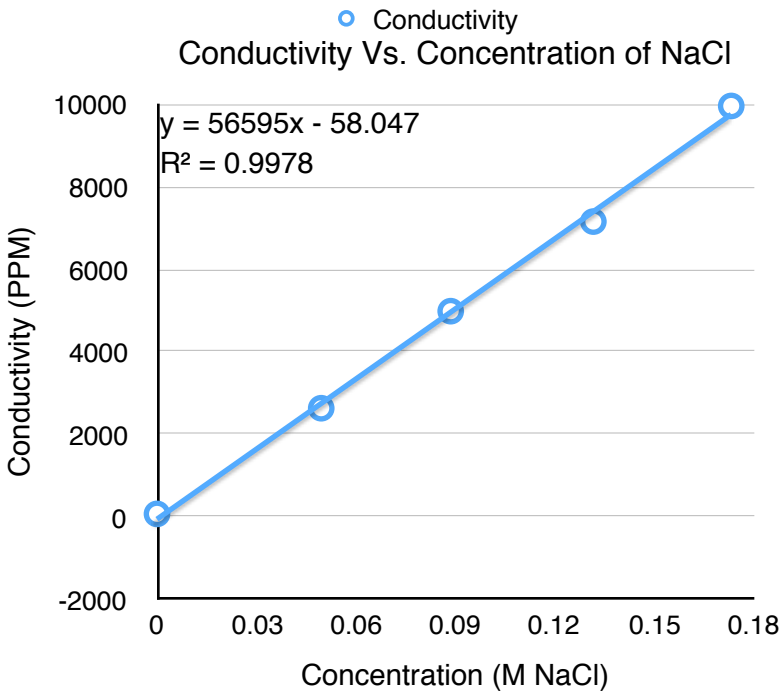

Figure 15: Standard curve for conductivity vs. concentration of NaCl

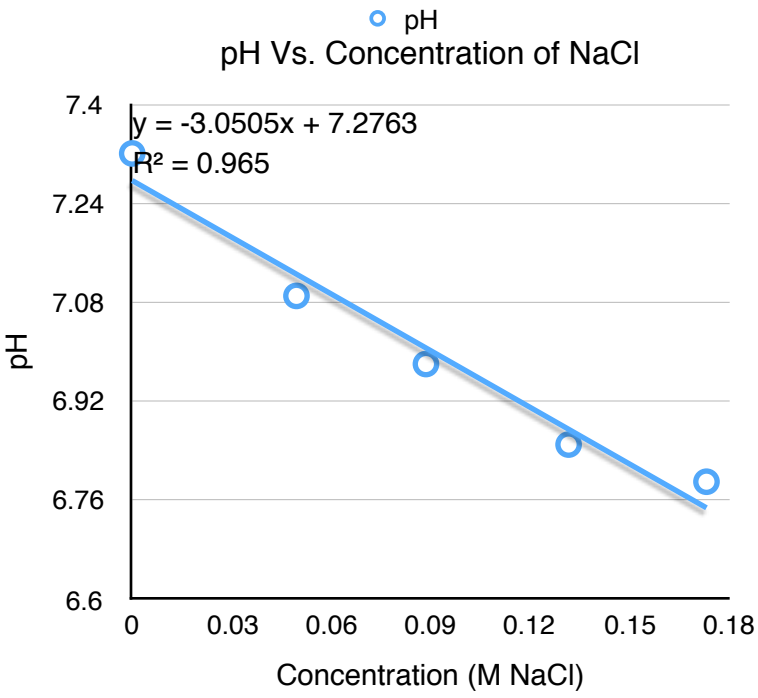

Figure 16: Standard curve for pH vs. concentration of NaCl

Flow Characteristics

Lower Channel Flow Characteristics		
Average Flow Rate (cm^3/s)	**Average Velocity (cm/s)**	**Average Mass Flow Rate (g/s)**
0.302	241.6	0.302
Min Flow Rate	**Min Velocity**	**Min Mass Flow Rate**
0.25	200	0.25
Max Flow Rate	**Max Velocity**	**Max Mass Flow Rate**
0.35	280	0.35

Table 3: Lower channel flow characteristics of microfluidic chip

Upper Channel Flow Characteristics		
Average Flow Rate (cm^3/s)	**Average Velocity (cm/s)**	**Average Mass Flow Rate (g/s)**
0.553	442.7	0.553
Min Flow Rate	**Min Velocity**	**Min Mass Flow Rate**
0.428	342.4	0.428
Max Flow Rate	**Max Velocity**	**Max Mass Flow Rate**
0.636	508.8	0.636

Table 4: Upper channel flow characteristics of microfluidic chip

Unrestricted Flow Characteristics of Continuous microfluidic device		
Volume Filled (mL)	**Time to Fill volume (seconds)**	**Flow Rate (mL/s) or (cm^3/s)**
8	12	0.667
8	**11**	0.727
8	10	0.800
8	10	0.800
Average:	10.75	0.744

Table 5: Unrestricted flow characteristics of continuous microfluidic device being developed (No microfluidic chip placed between input and output reservoirs, so flow is unobstructed). These demonstrate maximum values, which can be decreased by adjusting the height of the input reservoirs.

Transfer Results

NaCl transfer results						
Date	Time	pH (Reservoir 1)	pH (Reservoir 2)	Conductivity (Reservoir 1)	Conductivity (Reservoir 2)	Hours Elapsed
Dec 6	6:32 PM	6.82	6.71	69	3630	0
Dec 6	6:35 PM	6.85	6.71	69	1370	0.05
Dec 6	7:05 PM	6.85	6.74	69	1790	0.5
Dec 6	8:00 PM	6.83	6.77	68	1890	1.5
Dec 6	10:11 PM	6.87	6.75	69	1900	3.5
Dec 7	7:27 AM	7.03	6.90	70	1940	12
Dec 7	9:35 AM	6.98	6.93	70	1900	14
Dec 7	3:27 PM	7.06	6.92	72	1930	21

Table 6: Data collected during NaCl transfer trial. Total time of this trial was 21 hours. No significant transfer was seen from reservoir 2 (NaCl solution) to reservoir 1 (distilled water). Initial changes in conductivity in reservoir 2 is expected and is due to the system initialization process which is covered in the discussion section.

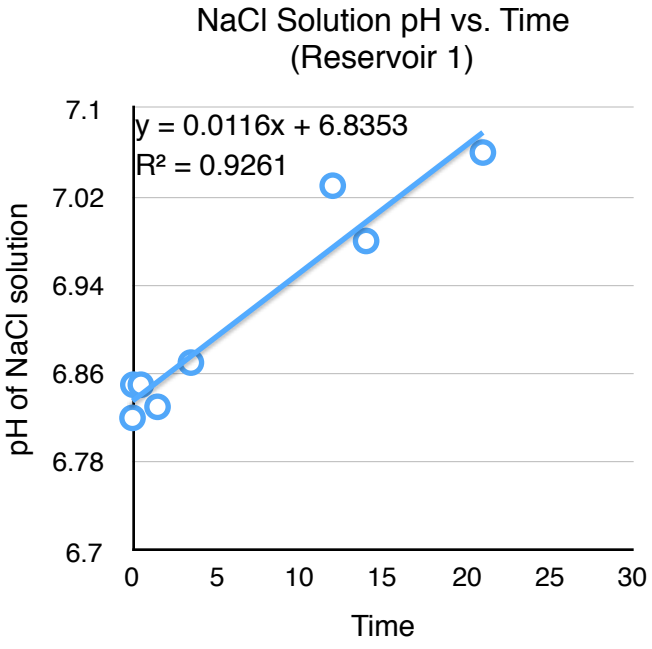

Figure 17: Change of pH in solution of reservoir 1 over 21 hours

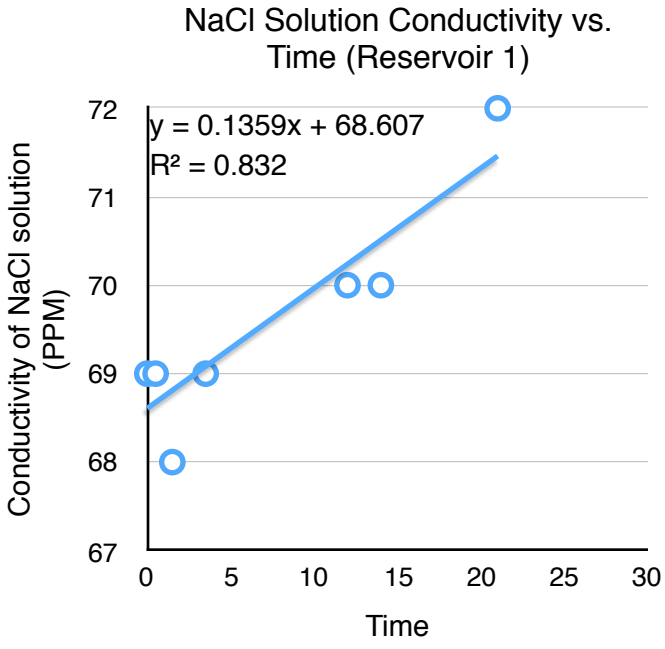

Figure 18: Change of Conductivity in solution of reservoir 1 over 21 hours

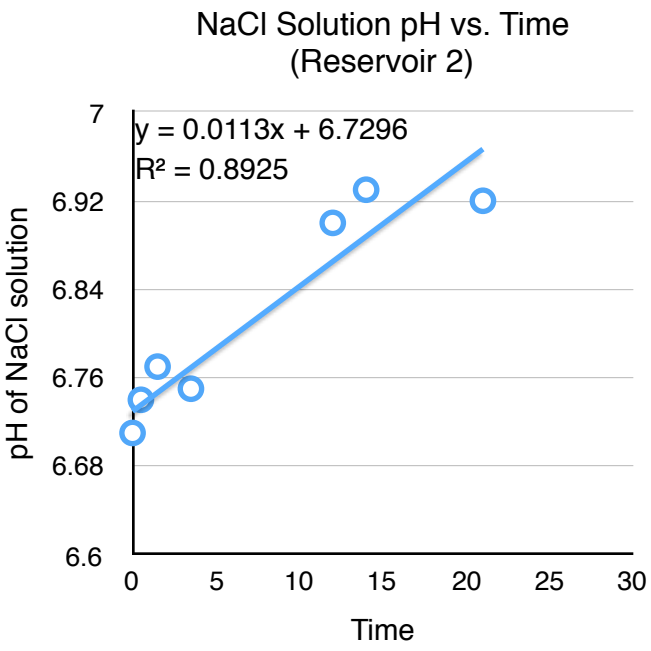

Figure 19: Change of pH in solution of reservoir 2 over 21 hours

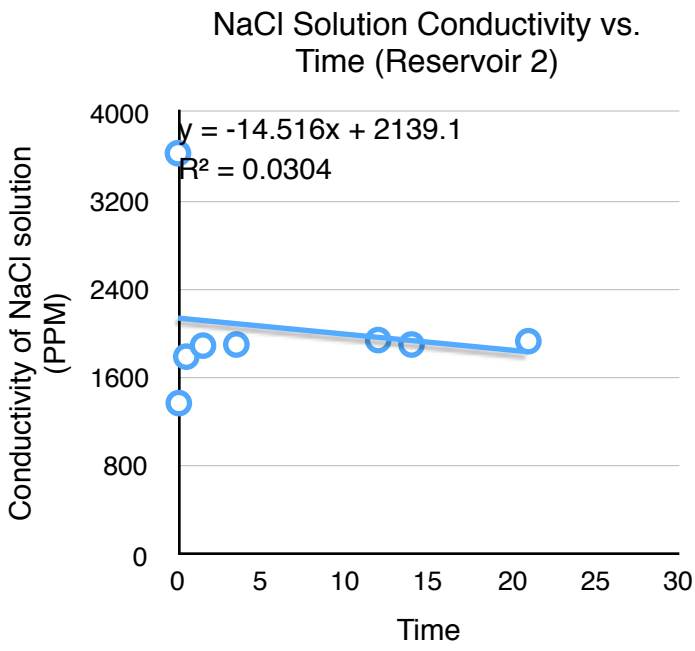

Figure 20: Change of Conductivity in solution of reservoir 2 over 21 hours

Discussion

The microfluidic chip and methods of testing being developed in this paper have shown very promising results although it still requires various tests to verify the flow characteristics of the chip and how useful this is as a model of the placenta membrane in vivo. The results of the preliminary tests show that the chip's fluid flow is correctly flowing from the input to the output, which can be seen in figure 11 and figure 12. Figure 11 shows that the alignment of the channels are correct and figure 12 shows that the color dye is staying within these channels. Upon microscope visual inspection, both channels, individually, seemed to show no evidence of flow outside of channels or around placenta membrane. Therefore, each channel did what it was supposed to with the flow characteristics shown above. A research paper from A. Stucker gave evidence that the mean capillary blood velocity was found to be .047cm/s [4]. Given a capillary

diameter of 3.7 microns, which gives a cross sectional area of 1.07e-7 cm^2, we can calculate the flow rate using the equation Q=VA. The flow rate, using data from Stucker's research paper, was found to be 5.02e-9 cm^3/s. In the "Placenta On A Chip" paper the flow rate being injected into the input has a value of 3.0e-5 cm^3/s [1]. Both of these flow rates are much less than that found in each of the channels tested, which means that using a device which can accurately control the fluid flow into the channels we can recreate and simulate flow velocities found in the human body. While our values of flow rate are much higher than this, it is easier to slow the speed down than to build a system to speed this flow velocity up. While the first set of results were based on qualitative values and relied heavily on observation to understand what was happening in the microfluidic chip, it gives a pretty clear picture that it is functioning as it is supposed to in terms of flow direction and containment.

Testing the transfer of different molecules in an aqueous solution proved to be a difficult problem for a few reasons. The first setup using the microfluidic pumps could only run for about 5 minutes per trial while "Placenta On A Chip" ran each trial for 68 hours [1]. This is a very significant different in time and could have meant that any transfer that did occur in that time was on a scale too small to measure with the available instrumentation.

Blue Dye Transfer Test

The first tests using food coloring (blue) showed no transfer in any of the trials, which is why the standard curve of blue dye concentration, seen in figures 12 and 13, is not followed by any conclusive results. The spectrophotometer measured no change in the absorbance of the output channel, meaning that there was very little or no transfer

at all. Upon further research it was found that the food coloring was actually a fairly large hydrocarbon chain which could have also been the reason that no transfer was seen.

NaCl Testing

The standard curves showed that there is a very strong negative correlation between pH and NaCl concentration and a strong positive correlation between conductivity and NaCl concentration. This can be seen in figure 15 and 16 which show R^2 values of .9978 and .965, respectively. This means that there is a very strong correlation between these parameters and that they can be used as an accurate indicator of concentration of NaCl in solution.

The first successful trial, table 6, of the NaCl tests showed that no transfer was seen over a period of 21 hours. While figure 18 shows an increase in conductivity from 69 PPM to 72 PPM, this relates to a change of molarity of .000053 M. This is most likely due to the accuracy limitations of the conductivity meter being that its resolution is only +/- 10 PPM. The change in conductivity of reservoir 2 seen in the first three data points makes sense due to how the initialization of the system occurs: The output reservoirs are filled with the corresponding solutions and as the pumps fill the input reservoirs additional distilled water is added to the output reservoirs until an equilibrium in the system is reached. This is consistent with what is seen in the first three data points, as the conductivity is starts very high, drops suddenly, and then levels off.

Although previous attempts to run experimental trials did not show any transfer across the placenta, future work will be aimed at determining why no transfer was seen. Specifically, to determine whether it was the design of the microfluidic chip or the

possibility of the amnion membrane being rendered impermeable during the processing of the membrane by MiMedx, who is the company supplying the placenta membrane.

Conclusion/Future Work

The microfluidic chip to test the amnion membrane has shown signs of being a successful model for an in-vivo placenta membrane although further testing is required to verify this. The simplified methods of constructing the chip using laser-cut PDMS, silicone tape, and acrylic has shown to be a useful and effective alternative to the photolithography method commonly used. In terms of cost, ease of manufacturing, and completion time our microfluidic chip is superior to that of the one described in “Placenta-On-A-Chip”. The comparison of flow characteristics in this new microfluidic chip to that of previous models is yet to be determined. As for the development and testing of the microfluidic chip, future iterations should represent the environment found in the body more closely. Once the chip is functional and provides a test platform closely representing the amnion membrane, the possibilities for using this device to study disease, nutrition, and characteristics of the placenta membrane would be huge and could give a better understanding of how this membrane works.

Once the lack of transfer of solution across the membrane is determined and corrected, this project will focus mainly on the molecules for testing. Testing other molecules that cannot have their concentration measured with pH or conductivity will require slightly new methods and materials, but can be modified from the current setup. Specifically, NaCl will continue to be the molecule of choice for tested because of its importance in bodily functions as well as the sensors already present in the continuous microfluidic device.

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