MICROFLUIDIC DEVICES TO STUDY NEURONAL AXON NETWORKS IN A BIOMIMETIC ALZHEIMER MILIEU

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Abstract

Alzheimer's disease, resulting from abnormal protein build-up in the brain, is detrimental to a patient's neuronal network and impacts millions of individuals within the United States. The research purpose of studying Alzheimer's disease is relevant because studying the Alzheimer's milieu will better inform future studies performed by researchers and increase therapeutic interventions within the healthcare industry. Moreover, creating a device that simulates the Alzheimer's microenvironment would allow for testing and manipulation of cholinergic neurons to generate conversance about how healthy neurons are affected by this disease. The final device consists of circular ports to house neuron somas, long thinning channels for unidirectional neuron axon growth, thin diffusion lumens for media/nutrient delivery, a main rectangular channel to mimic the Alzheimer's milieu, and inlets and outlets to promote flow throughout the device. Due to fabrication difficulties, the benchtop results from testing this device were limited, but COMSOL simulations validated the expected results. In the future, more extensive testing should be conducted, including neuron testing with the use of functional recording methods, such as microelectrode arrays and external electrodes. This will properly determine if the microfluidics device recapitulates the 3D structure of the physiological environment present within Alzheimer's disease and thus if it will enable high efficacy investigations of the Alzheimer's milieu.

Introduction

Over six million Americans have Alzheimer's disease, and the percentage of those with Alzheimer's increases with age. It is currently estimated that 500,000 new cases of Alzheimer's disease will be diagnosed this year. The cases for Alzheimer's are continuing to rise, there is no cure, and therapeutic intervention efficacy is low, demonstrating the need to properly understand and treat the pathology [1]. Alzheimer's is thought to be a result of an abnormal, toxic protein build-up in and around brain cells [2]. Therefore, as the disease progresses, cholinergic neurons become increasingly damaged and aberrant, eventually inducing their death. This will in turn cause brain regions to shrink, generating a significant loss in brain volume [3]. In addition to Alzheimer's large effect on memory, it impacts sight and language [2]. Alzheimer's is thus a dire pathology that reduces the quality of life of a significant amount of individuals.

Despite the impact Alzheimer's has, currently, there is a knowledge gap associated with the mechanisms Alzheimer's uses to create aberrant neuronal behavior and ultimately destroy axonal networks as well as key pathological features and signaling molecules [3]. More models that provide highly mimetic simulations of the *in vivo* Alzheimer milieu are thus needed in order to generate conversance about Alzheimer's and how to effectively treat it. As a result of this, the goal was to create a microfluidic device that promotes neuron growth and mimics the Alzheimer's microenvironment with a high degree of accuracy. This model will allow researchers to determine how healthy neurons are thus affected by the synthetically created environment, as well as several other factors if warranted. The objective is to create a device that can hold and unidirectionally grow neurons, so the size of the device must ergonomically match this by securing the neuron somas in place and encouraging elongation of the neuron axons and dendrites. It must also be capable of acclimating to changes in the neurons. A microdevice is useful to study an Alzheimer-like milieu as the microscale enables a high degree of mimicry of the *in vivo* environment due to the dimension match. The pathology also impacts neurons and a microdevice is the proper size to interface with, house, and guide cell growth of neurons. This will

allow individual studying and manipulation of neurons within an Alzheimer milieu, promoting proper investigation of the *in vivo* Alzheimer environment and thus generating pathologic conversance.

Methods

Fabrication Methods

An incremental design development and fabrication process was performed in order to establish a microfluidics device capable of properly mimicking the *in vivo* Alzheimer's disease milieu. Following the seeding of cholinergic neurons into the device, conversance will be generated about how Alzheimer's disease influences neurons, the aberrant neuronal behavior that is induced, pathological progression, and potential treatment mechanisms. To begin fabrication, a microfluidics device was developed within Adobe Illustrator and consisted of three main functional groups: 14 circular ports connected to long thin channels, 16 thin lumens, and a larger, rectangular main channel (see **Figure 1** below). To find the optimal dimensions to promote growth of neuronal axons into the main rectangle channel, diffusion of appropriate nutrients, media, and factors into the main channel and circular ports, and mimicry of neuronal network interactions, seven microfluidics devices were developed with various sizes and dimensions for each main feature. A standard model design for the microfluidics device can be seen in **Figure 1** below. The dimensionally altered models can be seen in the Appendix.

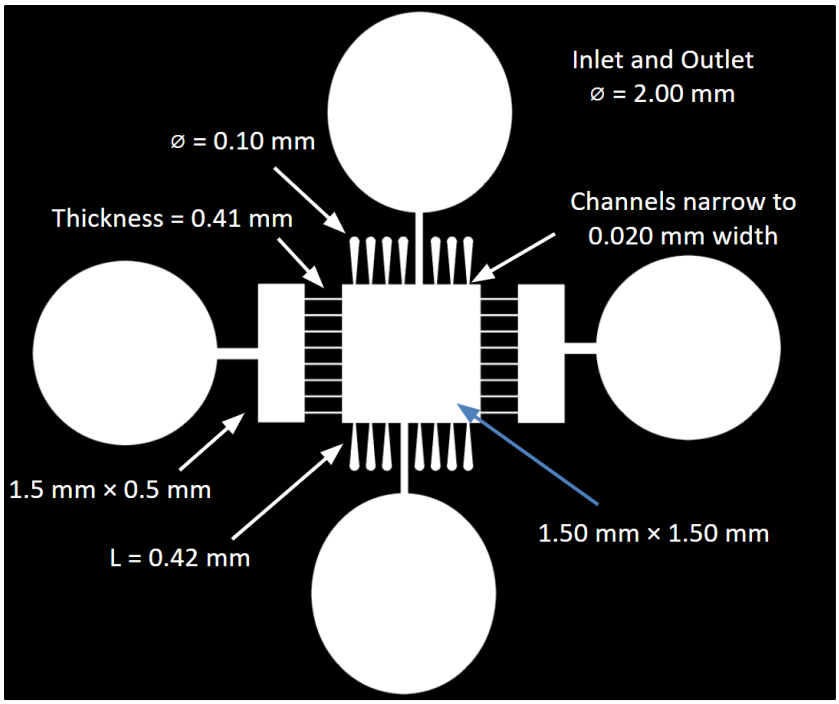

Figure 1: Adobe Illustrator depiction of the standard sized Alzheimer's mimicking microfluidic device. It contains circular ports to house the soma of the neurons, long thinning channels for unidirectional neuron axon growth, thin diffusion lumens for introducing media, nutrients, and factors, a main rectangular channel to mimic the Alzheimer milieu and promote axon interaction, and inlet (circular and rectangular sections on the lateral sides of the design) and outlet (large circular regions located at the superior and inferior sides of the design) ports.

The proceeding discussion about the microfluidics device will be based on the specific dimensions of the standard model seen in **Figure 1** above, however, the information, procedures, and justifications provided are also applicable to the other six devices and their dimensions. Each functional group was designed for a specified purpose in order to enhance the Alzheimer environment mimicry. The circular ports act as reservoirs for cholinergic neurons to be deposited into. The environmental conditions (chemical and mechanical properties) of these ports will promote the viability and growth of the cholinergic neurons, enabling Alzheimer's influence on neurons to be properly studied. The milieu will specifically consist of a 1 - 4 kPa surface stiffness and Advanced Dulbecco's Medium Modified Eagle Medium, 1 mM of β-mercaptoethanol, and 100 ng/ml nerve growth factor (NGF) [4, 5]. The 100 µm diameter size of each port will be large enough to house the neuron cell bodies as well as tight enough to secure each neuron in place within their respective reservoir, preventing soma transport down the

channels, due to their 40 μm diameter [6]. The long channels that extend within these ports will taper from 100 μm at the proximal end (closest to reservoir) to 20 μm, developing a pressure gradient. With the assistance of passive pumping, this will induce the flow of neuron growth, specifically in terms of their dendrite and axon extension, along the channels [7]. As the channels connect to the main rectangular 1.50 mm x 1.50 mm channel, the axons will be able to disperse throughout the main compartment, interacting with other neurons, simulating the neuronal network found in the forebrain. This interaction in the main channel is critical to the study of Alzheimer's, as the pathology damages cholinergic neurons, disrupting and altering their electrical interactions, resulting in memory loss [8]. The central location (1.50 mm x 1.50 mm chamber) will mimic the *in vivo* milieu that corresponds to Alzheimer's, containing a high concentration of β-amyloid peptides and highly phosphorylated tau proteins, enabling an analysis to be performed on the interactions between neurons [9]. As a typical cholinergic neuron has an average axon length of 107 mm with a density of 0.080 $\mu g/\mu m^3$ and a width of ~ 1 μm, the 1.50 mm x 1.50 mm size of the main channel is required to enable proper axon growth throughout the system and promote the neuron interactions that are vital to developing conversance about Alzheimer's [10]. The 16 thin lumen channels will be used to introduce media, nutrients, and factors into the system. They connect directly to the large main channel. The channels use passive pumping to diffuse all the required solutions and materials (neuronal growth stimulating media, β-amyloid peptides, and highly phosphorylated tau proteins) into the device, flowing into the main channel as well as through the axon channels and into the neuron ports [11]. Surface tension and adhesion forces will support this travel [12]. The compounds injected into the system will thus promote the development of an Alzheimer's environment in the main channel and cholinergic neuronal growth in the circular port reservoirs. Of note, the 2 mm diameter circular and 1.50 × 0.50 mm rectangular sections on the lateral sides of the design are the inlet ports and enable the required media, factors, and nutrients to be delivered throughout the system. The outlet ports are the large 2 mm diameter circular regions located at the superior and inferior sides of the design, collecting waste and old media within it. The solution in the circular neuron reservoir ports as well as the outlets will be removed periodically in order to dispose of waste and promote a constant flow into the channels.

A mask containing the seven Adobe Illustrator microfluidics device designs was ordered (see **Figure 2** below) and SU-8 photoresist photolithography was subsequently utilized to develop the patterns on a silicon wafer. To prepare the silicon wafer for pattern coating, it was exposed to air in order to clean its surface and remove any residual dust and particles. The presence of extraneous material on the surface of the wafer will generate an uneven pattern that will limit the success of the microdevice. As a result, this is a crucial step. Following the cleaning of the silicon wafer, it was pre-baked for ten minutes (65^{o}C) followed by a two minute cool-down in preparation for SU-8 photoresist coating.

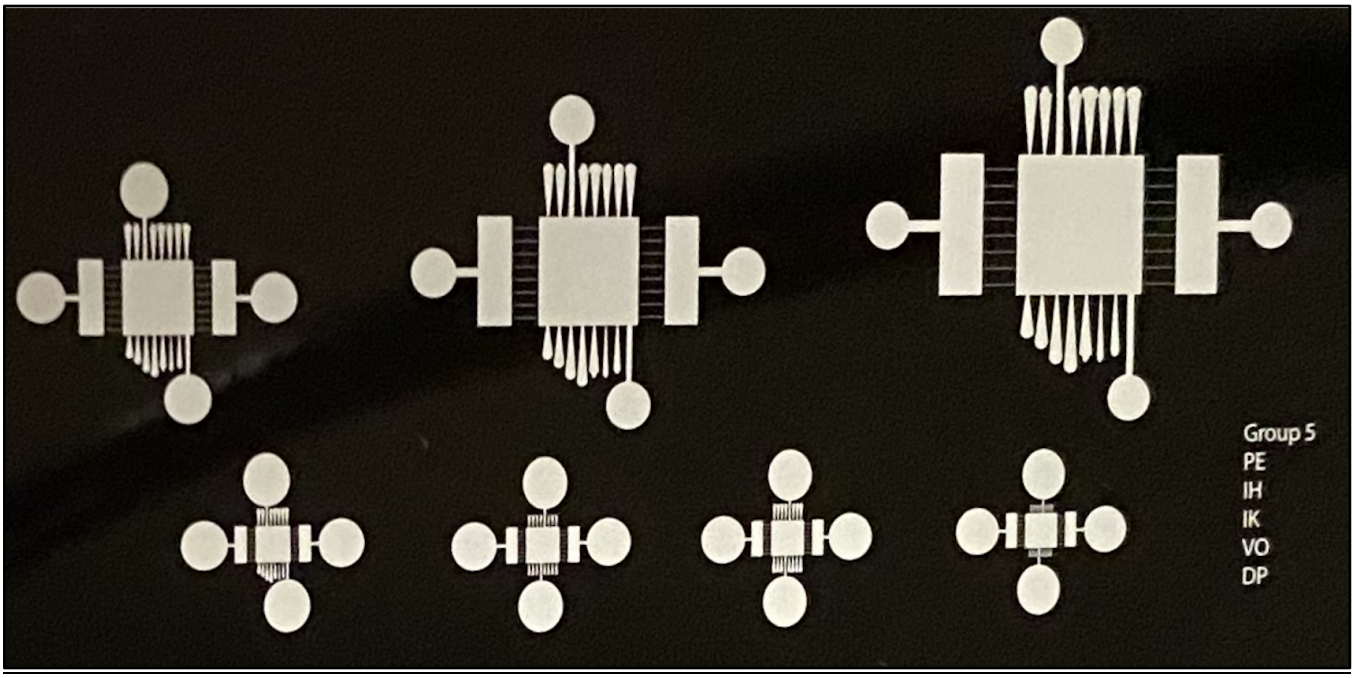

Figure 2: Photomask containing the seven Alzheimer mimicking microfluidic devices, each with varied dimensions to establish the optimal size for each design feature in order to promote Alzheimer milieu mimicry and thus pathology conversance.

After the wafer was appropriately cool-downed, it was placed on a spin-coating system. To verify that the wafer was in the correct location on the device, a test spin was performed, ensuring the silicon wafer stayed in the center of the system. The silicon was deemed ready for photoresist coating when the tests confirmed the success of the wafer-spinning system. To ensure UV light exposure can generate the desired pattern on the wafer with deep enough channels to enable proper diffusion and flow dynamics, the

SU-8 photoresist must be homogeneously distributed across the wafer with a thickness of ~ 120 µm. This thickness will also promote the longevity of the wafer and subsequent PDMS mold device. The SU-8 is thus carefully poured onto the middle of the spinning wafer and spun at a 1000 rpm rate for 40 seconds. This rate and duration resulted in the spin coating procedure generating a ~ 120 µm thick SU-8 sheet onto the wafer as a result of its 65°C pre-bake. To properly develop and solidify this coat, the wafer-SU-8 photoresist complex underwent a 30 minute, 95°C softbake, followed by a two minute cool-down [13]. The resulting SU-8 photoresist coated silicon wafer can be seen in **Figure 3** below.

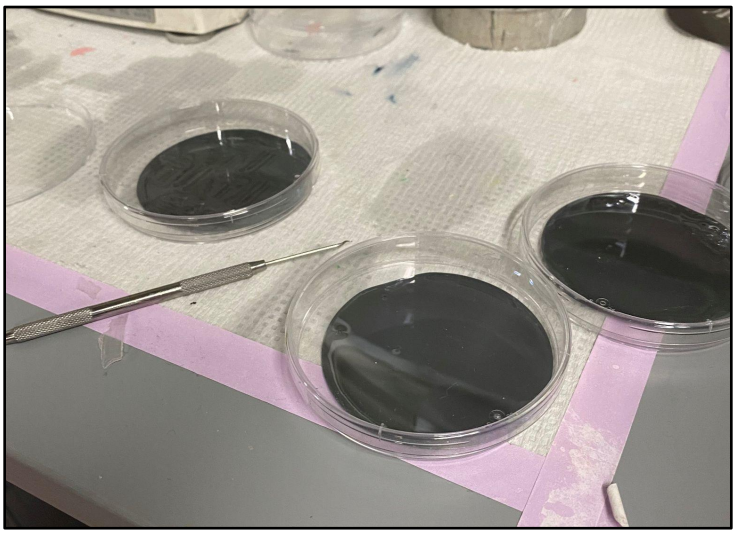

Figure 3: Silicon Wafers coated with a ~ 120 µm thick SU-8 sheet following their 30 minute 95°C softbake.

With the wafer coated in the SU-8 photoresist, the next step was to expose the silicon to 365 nm UV light through the patterned photomask (see **Figure 2** above) in order to generate the appropriate topology on the photoresist. As SU-8 is a negative photoresist, all material illuminated by the UV light will solidify and remain following development, while the unexposed regions will dissolve. To prepare the UV light for exposure to the wafer and SU-8 photoresist, a four minute warm-up period was required for the light. The power of the UV light was then taken to determine the required time in order to provide the desired 360 mJ/cm^2 exposure energy to the photomask-SU-8-wafer system and thus fabricate the required pattern on the wafer. Initially, with a power of 14180 W, the wafer was exposed to 365 nm UV

light for 27 seconds. Unfortunately, after a post-exposure bake and development, this duration overexposed the wafer, resulting in microfluidics devices that contained blurry edges and features that were merged together. The devices did not have the resolution required for proper diffusion, transport, and growth to take place within the system in order to mimic the *in vivo* Alzheimer environment to a high degree. To solve this issue, a new SU-8 coated wafer was developed following the aforementioned process and was then exposed to UV light through the pattern photomask for only 25 seconds, opposed to the previous 27 seconds. Although this reduced the exposure energy provided to the photoresist, this enabled appropriate feature development on the wafer. With the pattern now coated into the SU-8 photoresist, the wafer underwent a post-exposure bake of 65ºC for one minute and 95ºC for ten minutes [13]. This procedure prepared the wafer for development of the desired pattern and can be seen in **Figure 4** below.

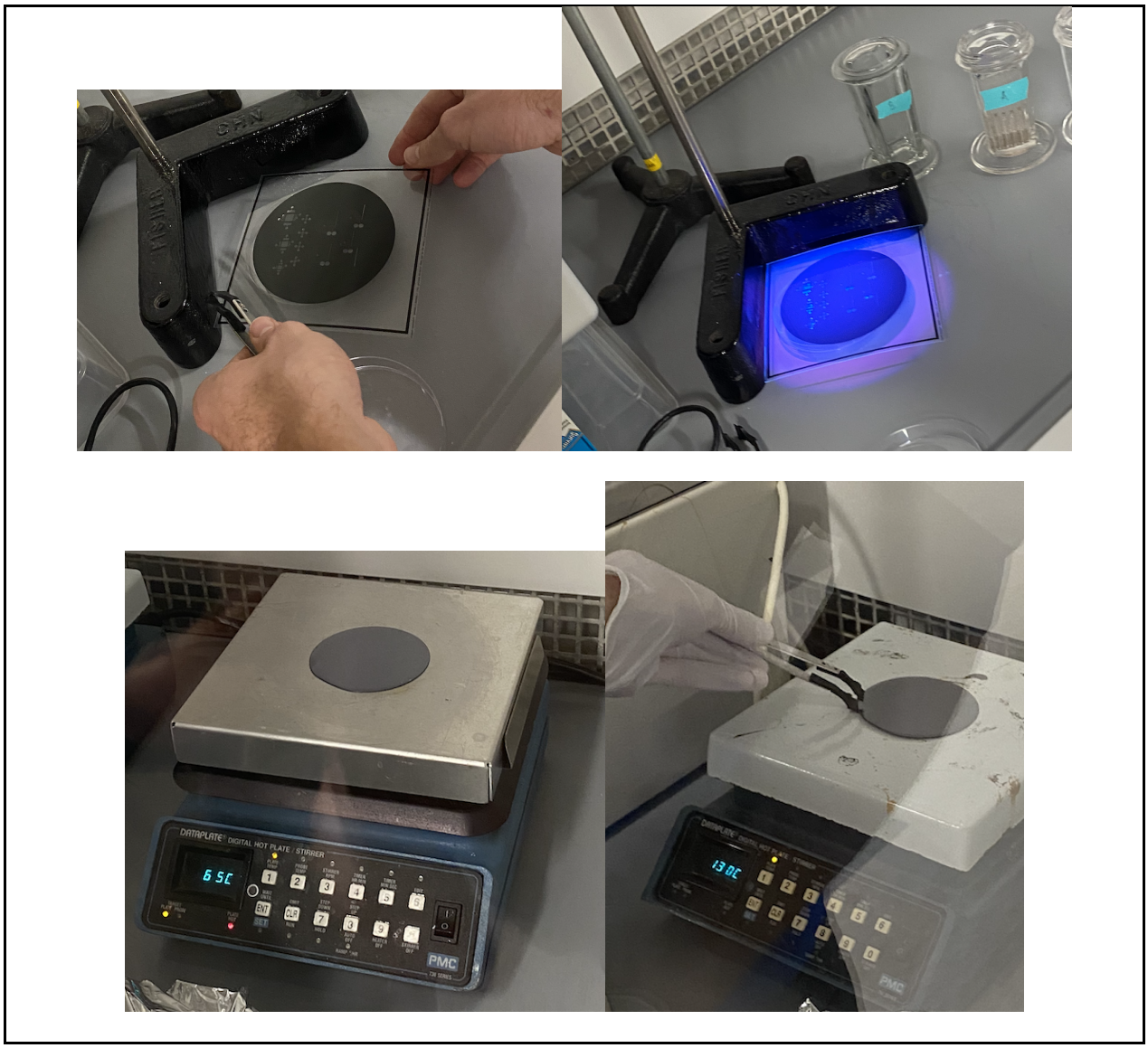

Figure 4: Proper alignment of the photomask over the SU-8 coated silicon wafer (top left) and the subsequent illumination of the wafer through the photomask via 365 nm UV light in order to develop desired micropatterns within the wafer (top right). The bottom figures display the post-exposure bakes, with the initial one minute, 65ºC bake depicted in the bottom left and the ten minute, 95ºC bake illustrated in the bottom right.

Following the completion of the post-exposure bake, the wafer was placed into SU-8 developer (a non-toxic synthetic compound) for ten minutes in order to develop the mask pattern on the silicon wafer. This process took place within a beaker covered with aluminum foil, minimizing contamination risk. The wafer was checked at three minutes and seven minutes to track the progress of development and determine if the wafer is ready ahead of time, if it will require the full ten minutes, or if the wafer will

need to develop for longer than ten minutes. The wafer could only be removed from the developer for a minimal amount of time when checking the progress, as prolonged periods of time removed from the developer will dry the wafer out and prevent successful pattern development. The pattern took ten minutes to develop, generating the desired high resolution microfluidic devices. As SU-8 is a negative photoresist, the microfluidic device patterns were developed through the solidification of the areas exposed to UV light and dissolution of the regions that were hidden from the light. After proper development of the wafer, it was rinsed with isopropanol, followed sequentially by deionized water, in order to remove residual developer left on the superior surface of the wafer. The wafer was then dried with a gentle stream of nitrogen gas (via a nitrogen gas spray gun), finalizing the fabrication of the microfluidic device on the wafer [13]. The development of the SU-8-wafer and resulting product can be seen in **Figure 5** below.

Figure 5: Development of the SU-8 coat on the wafer within an aluminum foil covered beaker (left), the resulting patterned SU-8-wafer following development (middle), and the final wafer product after isopropanol rinsing and nitrogen gas drying (right).

Although the microfluidic device patterns have been generated on the wafer, it can not be utilized as an individual device as the pattern is only present on the wafer. As a result, PDMS molds replicating each microfluidic device were fabricated in order to obtain a device that can be tested and implemented for research. To fabricate the PDMS, a 10:1 ratio of Sylgard 184 silicone elastomer base and elastomer

curing agent were mixed together in a petri dish and then placed in a vacuum for an hour to degas the solution and remove bubbles. After the solution was properly degassed via the vacuum, it was removed from the chamber and the patterned silicon wafer was placed within the vacuum chamber for one hour. This enabled a complete removal of bubbles from the wafer, including within the microscale channels, in preparation for PDMS mold development of the wafer patterns. The removal of bubbles allows this PDMS mold generation to take place with minimal impurities, developing adequate microfluidic devices for testing and *in vitro* Alzheimer investigations. The preparation of the PDMS solution and vacuuming procedure can be seen in **Figure 6** below.

Figure 6: Preparation of the 10:1 ratio of Sylgard 184 silicone elastomer base and elastomer curing agent in order to generate the desired PDMS solution (top left) as well as a depiction of solution mixing (top right). The bottom figures display the vacuum chamber set-up (bottom left) utilized to degas the PDMS solution and wafers, the degassing of the PDMS (bottom middle), and the subsequent bubble removal of the wafers (bottom right).

Following proper bubble removal from the silicon wafer, the wafer was taken out of the vacuum and coated with the PDMS mixture (10:1 ratio of Sylgard 184 silicone elastomer base and elastomer curing agent). The PDMS solution was carefully poured over the wafer, ensuring a homogenous distribution of the mixture was obtained along each microfluidics device pattern on the silicon. With the completion of PDMS deposition, the wafer was cured for 2.5 hours at 80ºC and then underwent a ten minute cool-down. As the PDMS was cured within a plastic petri dish, the curing was limited to a

maximum temperature of 80°C due to the melting point of plastic [14]. The curing and cool-down process produced a high resolution, solidified, and mimetic PDMS microfluidics device mold that enables proper *in vitro* analysis of Alzheimer's. As there were seven different PDMS molds fabricated, one for each microfluidic device pattern, the molds were placed onto a glass plate and exposed to oxygen plasma for three minutes. This resulted in attaching the PDMS molds to the glass, creating a substrate for all of the molds to adhere to in order to increase the strength, durability, cyclic fatigue resistance, and portability of the devices as well as promote testing feasibility. Subsequent baking of the glass-PDMS construct at 65°C for ten minutes further strengthened the glass-PDMS bond, ensuring the substrate was tightly adhered to the molds. For ease of use during testing, the glass plate was split into two smaller regions, one substrate containing four microfluidic device patterns while the other plate contained three patterns. To finalize the microfluidic devices, the inlet and outlet 2 mm diameter ports were punched to allow delivery and removal of the necessary solution, media, nutrients, and factors. After punching holes into the PDMS, the devices were ready for testing to determine their efficacy in mimicking the *in vivo* Alzheimer's milieu and the promote axon growth from the neuron soma reservoirs into the main channel (see **Figure 1** above). The final PDMS products can be seen in **Figure 7** below.

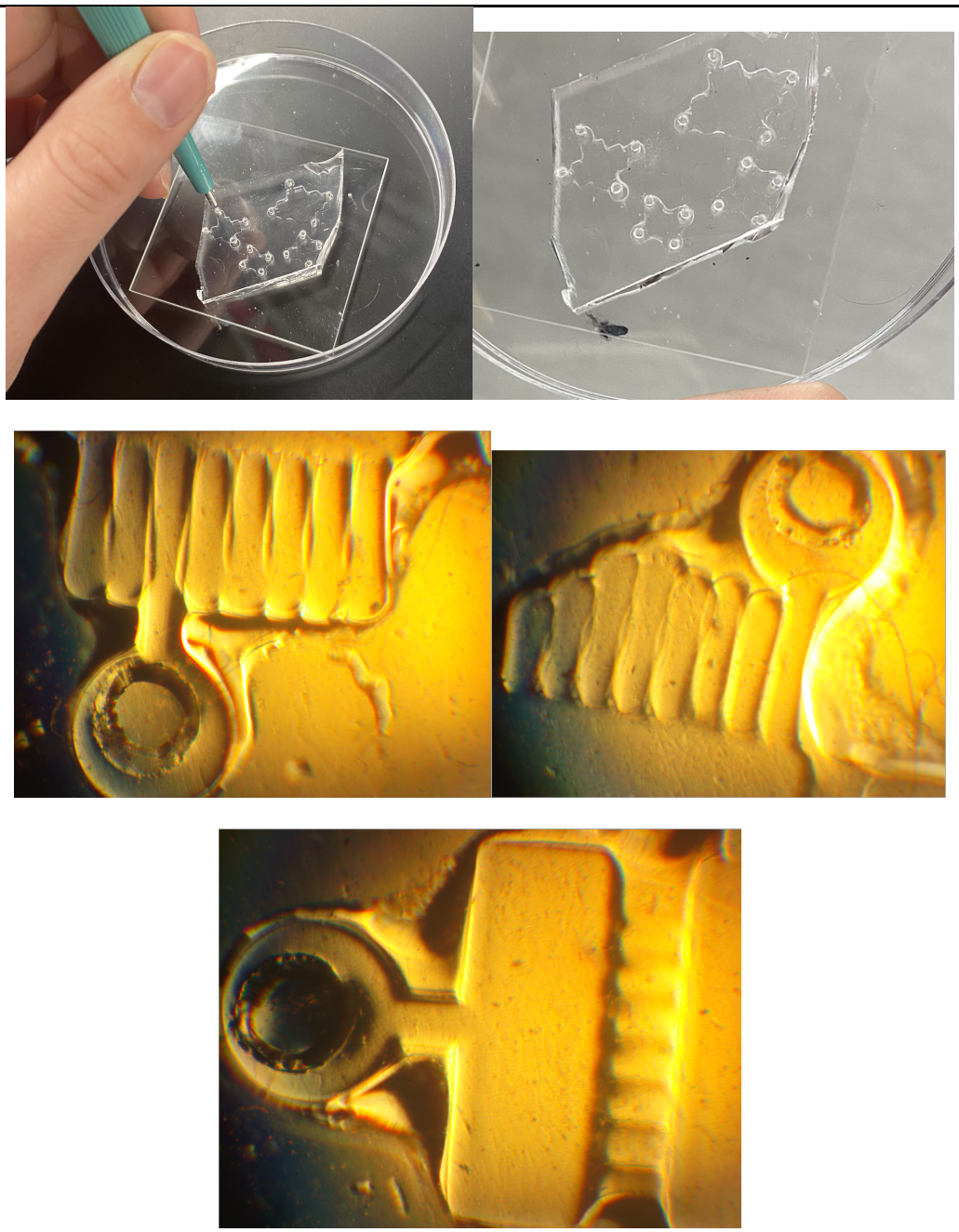

Figure 7: Illustration of punching the inlet and outlet holes for the PDMS microfluidic devices (top left) and one of the glass substrates containing the PDMS systems (top right). The middle left and middle right figures are inverted microscope depictions of the bottom and top neuron reservoirs and outlet holes, respectively. The bottom figure resembles an inverted microscopy illustration of the diffusion channels as well as inlet port and storage.

Validation Testing Methods

To validate the microfluidic device, an *in silico* COMSOL and a practical benchtop simulation tested the efficacy of the microfluidics device. The physical, practical testing established the functionality of the microdevice device using colored dye and a needle/syringe system. Both testing systems were utilized in order to determine the environment and flow patterns that would be created with the device. Primarily, these tests were used to confirm that the diffusion ports operated correctly and created a diffusion gradient, delivering the required media, nutrients, and factors to the main channel of the device where the neuronal axon network would be located. Altogether, testing was used to confirm the correct flow for cell growth and movement into the Alzheimer's mimicking milieu from the ports.

The *in silico* testing was performed using a COMSOL simulation. The Adobe Illustrator design was used as a reference to create a SolidWorks 3D model of the device (see **Figure 1** above) which was then imported into COMSOL for *in silico* testing. The SolidWorks 3D model had variations from the original design due to the size constraints of the SolidWorks software program. The micro-scale is too small for SolidWorks, so the Adobe Illustrator design was increased by a factor of ten in order to convert all dimensions to the milli-scale. The design of the diffusion channels were enlarged as they were too small to transfer over into SolidWorks as well as extrude properly. Additionally, the large outlet ports (see **Figure 1** above) were not in the same position as they were for the Adobe Illustrator design as the mirror tool was used in SolidWorks in order to simplify the system. Consequently, the superior and inferior sides of the microfluidics device are completely symmetric. The SolidWorks design was extruded to have a thickness of 50 mm so it would be approximately to scale with the thickness anticipated in the final PDMS mold. The final SolidWorks design with a scale bar can be seen in **Figure 8** below.

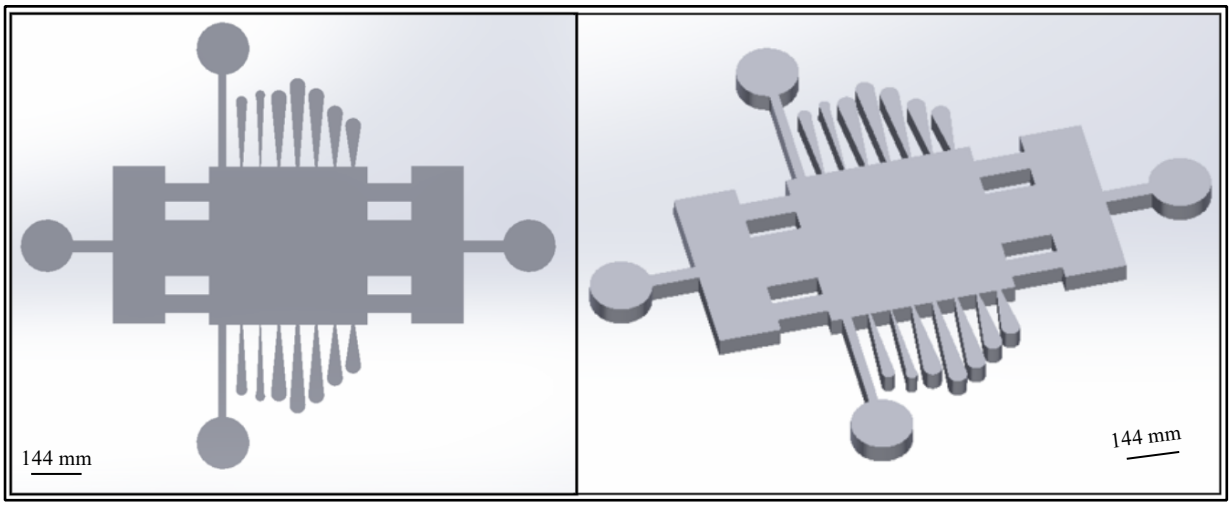

Figure 8: SolidWorks depiction of the standard sized Alzheimer's mimicking microfluidic device. Similar to the Adobe Illustrator design, it contains circular ports to house the soma of the neurons, long thinning channels, thin diffusion lumens, a main rectangular channel to mimic the Alzheimer milieu and promote axon interaction, and inlet / outlet ports.

The SolidWorks file was used to simulate the device in COMSOL. The SolidWorks file was imported into COMSOL and a series of stationary and time-dependent studies were performed. The parameters used include laminar flow, diluted species transport, and inlet and outlet assignment. This testing was performed to validate the flow that would occur through the device to ensure the media, the nutrients, and the factors were properly delivered to the main channel and somal chambers. While testing, it became apparent that for chambers to receive flow, they had to be defined as outlets. See **Figure 9** below for an example of one of the flow rate measurement tests that was performed.

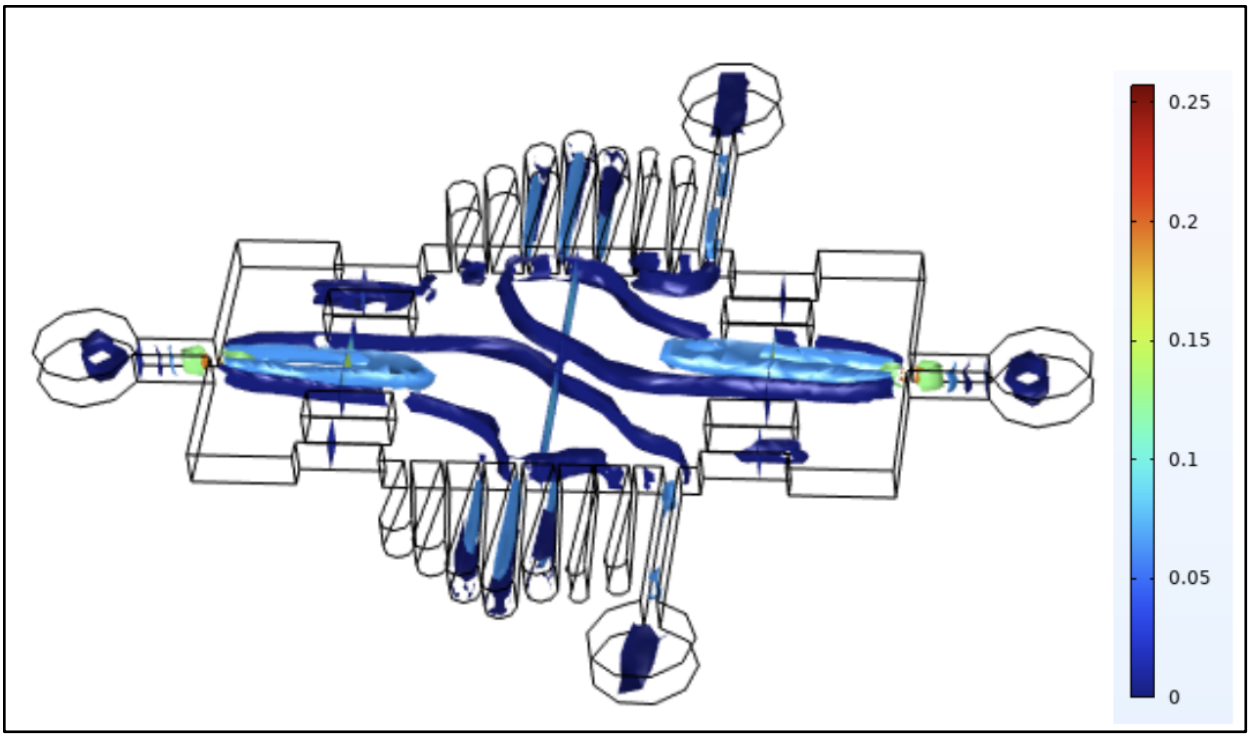

Figure 9: COMSOL model depiction of the standard sized Alzheimer's mimicking microfluidic device with three neuronal outlets on either side, two large outlets and two large inlets, and six diffusion channels.

Next, the practical, benchtop tests were performed to confirm the flow throughout the device. Diluted, colored food dye was injected via a syringe, tubing, and needle system into the inlets. The fluid was delivered via a hole in the inlet at an injection rate of ~0.025 mL/second. The needle went through the entirety of the PDMS up until the glass surface to ensure that the fluid would reach the location where the microdevice was contained. The injection and overall flow of the fluid was recorded and inspected using a Leica MZ95 Stereomicroscope at a 0.6x magnification in order to see the entirety of the device [15].

The device was first observed to determine how well the microdevice was fabricated. Unfortunately, it was determined that there was merging of the neuronal and diffusion channels which would interfere with some of the results given that the solution could flow through the system a lot easier when a few smaller channels fused into larger ones. In addition, there were further problems with low resolution in the smaller designs because most of the channels fused together to form one large

conglomeration. Therefore, the larger devices were the primary focus of testing to determine whether the proper nutrients traveled to the main channel (with the axonal network) as well as the different sized neuronal reservoirs (circular ports). Images and videos were taken using the Amscope software associated with the Leica MZ95 Stereomicroscope and the videos were analyzed in slow motion to determine if any conclusions could be made [15].

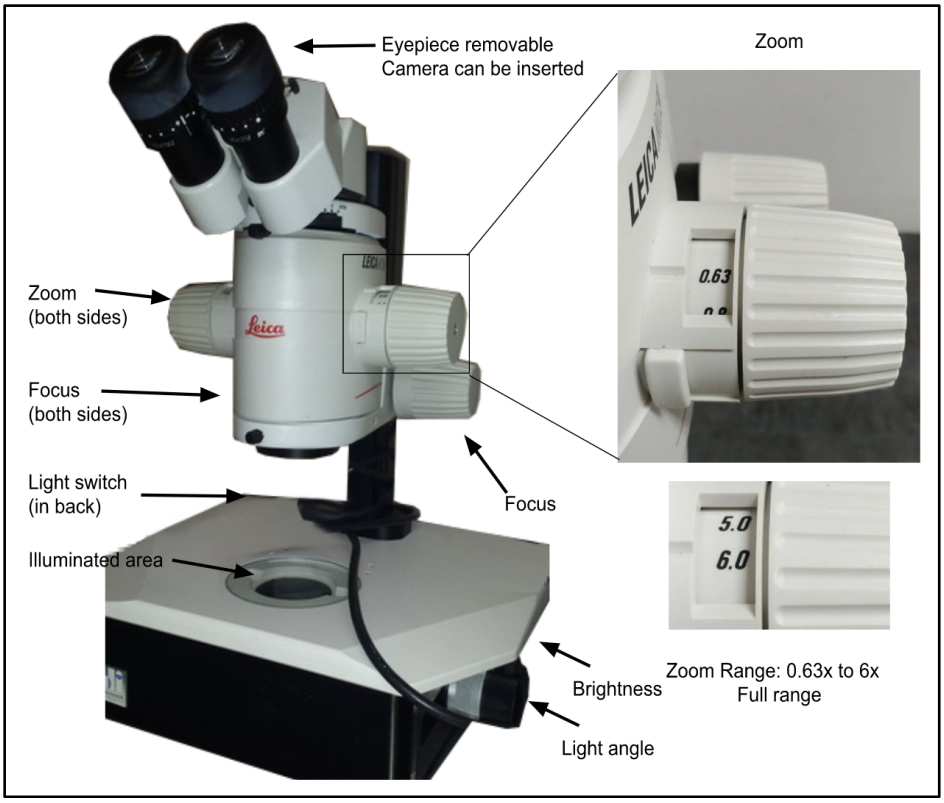

Figure 10: Leica MZ95 Stereomicroscope in the 1002 ECB Teaching Lab [15].

Colored food dye was used for testing in order to determine the flow of fluids throughout the device. The goal was to try and quantify the flow and concentration levels of the colored fluid as it dispersed throughout the system. Additionally, if neurons, neuronal media (see *Fabrication Methods* section above), and growth factors such as NGF were available, the device could have been tested by observing the fluid flow and growth of the neurons in the main channel [16]. The concentration of amyloid plaque, β-amyloid peptides, and highly phosphorylated tau proteins could have also been established during testing to determine if the Alzheimer's environment is becoming worse, suggesting a

progression of the disease and thus proper mimicry of the *in vivo* milieu [17]. Review of the proof-of-concept measurements and future testing can be found in the *Results* and *Discussion* sections, respectively.

Results

Proof-of-concept measurements: In Silico

To establish the efficacy of the microfluidics design, *in silico* testing was performed based on the outline provided in the *Validation Testing Methods* section above. The results displayed that injecting fluid at different flow rates (ranging from 0.0025 m/s to 0.025 m/s) from the outside walls of the inlets generated a uniform flow that covered the entirety of the device. This is crucial as this aims to serve as the input for media, factors, and future treatment agents (see *Methods* section above). The ability for the entire device, including the neuron reservoir circular ports, to receive the solution via injections at the inlets, confirms the success of the system.

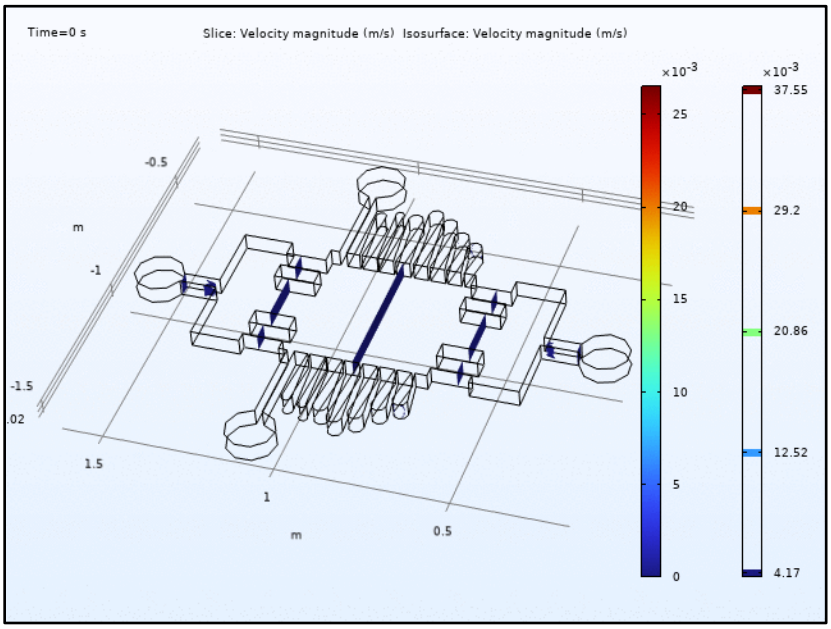

Figure 11: Velocity of flow throughout our device in a time dependent simulation. Injection flow rate was 0.0025 m/s. The animation's settings are 100 seconds with increments of 0.2 seconds, 10 fps with 250 frames total.

Pressure differences at the time of the injections were also investigated. As seen in **Figure 12** below, the highest pressure occurs next to the injection sites (inlet ports) and drops to its minimal value at the end of the defined outlets (outlet and neuronal reservoir ports). This is important to consider as this will impact the concentration of the injected agents, developing a concentration gradient leading into the somal and axonal locations. This variance can be utilized to tailor concentrations of specific materials, such as NGF, in order to promote axonal growth and neuron health will enable the device to provide proper *in vitro* investigations of the Azheimer milieu.

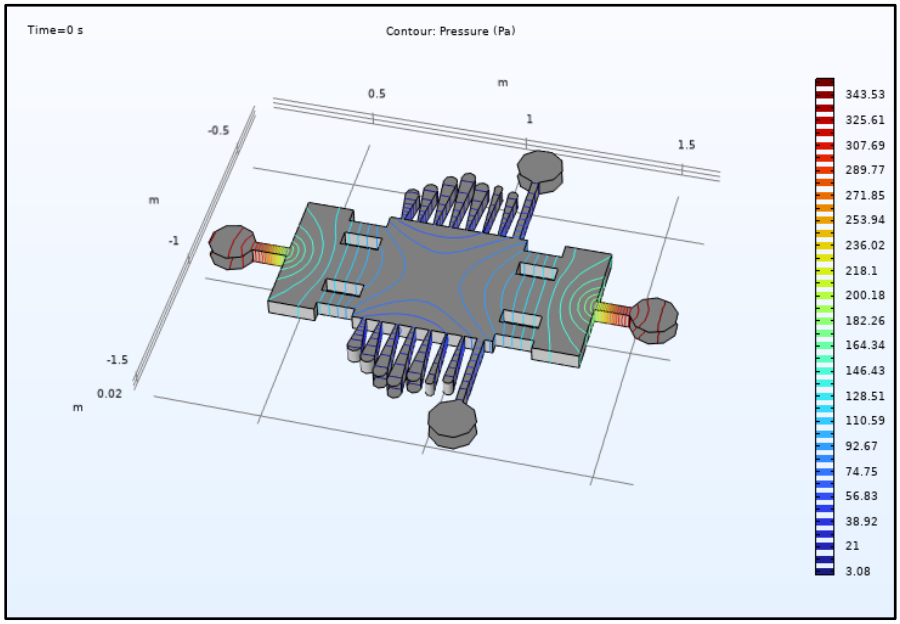

Figure 12: COMSOL simulation of pressure throughout the device.

Proof-of-concept measurements: Benchtop

As a majority of the microdevice's channels were fused together, finding an efficient and effective way to show the full functionality of the device was a challenge. Problematic PDMS-glass plate attachment, low resolution patterns, and PDMS rupture during the fabrication process also limited the benchtop results. It is still important, however, to acknowledge the effort put into testing the functionality of the device. With that in mind, the benchtop testing was focused on showing that fluid flow took place

and that with the creation of a “perfect” device, a gradient would form as a result of the passive pumping injection of dyes into the device.

To show the functionality of fluid flow, yellow dye was injected into a single inlet for one of the microfluidic devices. Unfortunately, the PDMS did not adhere to the glass slide properly. Thus, in the first round of testing, there were not any conclusive results as the dye would consistently leak underneath. Therefore, the PDMS was adhered to the glass slide a second time, ensuring that it was secured properly. A second round of testing was then performed. The testing yielded much better results this time, although it could not be effectively quantified. They did show proof-of-concept in that it created a linear flow from injection site to the opposing injection and outlet ports with slight diffusion into the fused neuronal channels. The video linked here displays a successful validation of the diffusion and neuronal reservoir channels. It is important to note that fluid can be seen outside of the outline of the device due to previous testing and faulty PDMS attachment, however, it does not reduce the validation of the diffusion channels. In the future the device needs to be much more defined in order to provide high-resolution channels that display correct diffusion. The fluid should also be injected at the same speed throughout the testing process to provide consistency across trials. This will produce data that can be analyzed without requiring normalization, reducing the potential for error.

Discussion

Alzheimer's disease is thought to be caused by the abnormal buildup of proteins in and around brain cells. Two such proteins include amyloid and tau. Amyloid is thought to clump together into plaques in between the neurons and block the information flow [9]. In a brain with Alzheimer’s, chemical changes within the brain will cause the tau proteins on microtubules to dissociate then reassociate to other tau proteins. These chains of tau proteins continue to accumulate within neurons and block the neuron’s transport system, ultimately affecting synaptic transmission [3]. Unfortunately, there is minimal conversance about the mechanisms controlling these steps as well as the process that follows as part of Alzheimer’s disease. The device described above will allow delicate manipulation of the system’s environment in a controlled, experimental fashion in order to induce appropriate Alzheimer's mimicking

phenotypes in the cholinergic neurons. Recent studies have grown organoids to express Alzheimer's-like pathology by exposing them to healthy blood serum. Researchers have confirmed the protein called phosphatase 1 (PP1) acts as a molecular link between the genetic risk factors of late-onset Alzheimer's disease and the amyloid plaques and tau tangles [18]. With this in mind, experiments could be performed where different solutions are inserted into the device followed by the measurement of the amount of PP1 from the outlets at various time points after treatment. Standard growth media and blood serum could also be used as controls for new altered solutions that screen for factors suspected of contributing to Alzheimer's disease.

Based on the testing and results, the device should still function as intended. However, slight modifications in the fabrication process should be considered to bring the idea to fruition. The computer simulated flow data suggests the media and other reagents would properly reach the neurons as initially intended. So, it will be vital to ensure measures are taken to avoid further unnecessary problems of the microfluidic device. First thing to consider would be to spray the glass plate with ethanol to sterilize and completely dry the surface to increase the chance of proper attachment of the PDMS mold. Second, the amount of time spent exposing the photoresist as well as the amount of time the wafer and photoresist are in the developer need to be optimized. This will reduce the chance of the channels in the microfluidic device merging with each other and increase the longevity of the device by increasing the structural integrity of the thin walls. The devices will need to be sterilized and packaged using a glass coverslip to keep them uncontaminated and maintain their structural integrity. With these design adaptations, the device would provide a small scale working environment with the ability to produce large data sets in order to quantitatively measure and predict the outcomes of the Alzheimer's disease environment as well as understand the mechanisms and key features that mediate the progression of Alzheimer's.

Assuming the PDMS is sealed properly and quantitative fluid flow and media concentration tests are run successfully, it would be interesting to look further into performing functional recording on the cells of interest. One way this can be completed is through the use of micro-electrode arrays and external electrodes in order to study a closer representation of the true physiological 3D structure. With the current

device, the system is studied in 2D. This limitation can result in losing valuable information such as how blood flow contributes to the spread and build-up of amyloid and tau proteins. Lastly, assuming the device is successful in mimicking an Alzheimer's milieu, this device should be able to be used in the research of Alzheimer’s disease in order to generate understanding of the underlying mechanisms eliciting and progressing the disease as well as promote the development of novel treatments.

References

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Appendix

Adobe Illustrator Microfluidic Device Patterns

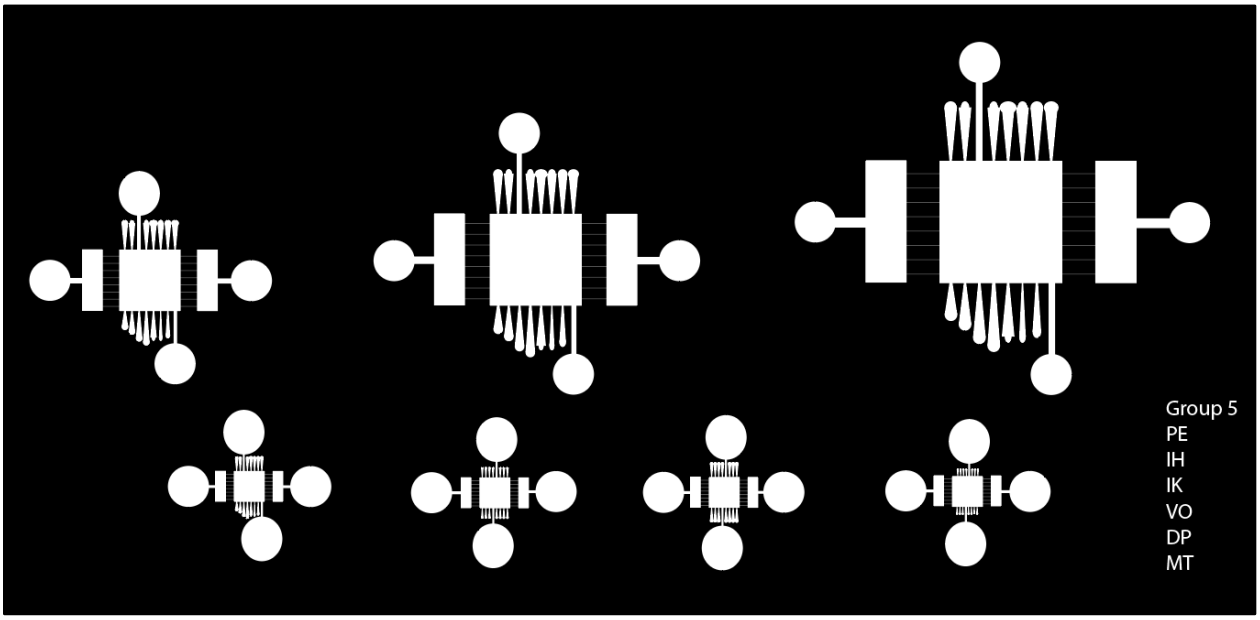

Figure S1: Adobe Illustrator depiction of the seven Alzheimer's mimicking microfluidic devices and their specific layout within the mask. They each contain circular ports to house the soma of the neurons, long thinning channels for unidirectional neuron axon growth, thin diffusion lumens for introducing media, nutrients, and factors, a main rectangular channel to mimic the Alzheimer milieu and promote axon interaction, and inlet (circular and rectangular sections on the lateral sides of the design) and outlet (large circular regions located at the superior and inferior sides of the design) ports.