MICROFLUIDIC SYSTEMS FOR THE STUDY OF THE CAENORHABDITIS ELEGANS WORM

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Two microfluidics devices are presented, which are used to further study of the Caenorhabditis elegans worm (*C. elegans*). The first device is a tool for ranking the muscle force between mutant and wild type strains of worms. The second device is a screening chip, which is designed to decrease the amount of time needed to screen the C. elegans worm, and does not require the use of anesthetics to immobilize them for imaging.

The muscle force tool operates by compressing a worm in a microchannel with a flexible Polydimethylsiloxane (PDMS) membrane. The force the membrane exerts on the worm is determined by air pressure controlled using a sensitive regulator. The method mimics the natural environment of the worm, where it must move through soil. Worms are tested by loading them into the chip and air pressure against the membrane is increased in increments until the worm becomes immobilized. To rank strains of worms according to muscle force, the pressures at which the worms become immobilized are compared. The chip operates on the hypothesis that a higher pressure indicates a greater muscle contraction force. The chip was tested using three strains of worms: a wild type and two strains with genetic knockouts of specific ion channels at the neuromuscular junction. Immobilization pressures are given for each strain.

The screening chip is designed to be operated on a confocal microscope, and is used for taking high magnification images and videos of the worms. To perform the

screening process, the chip separates a single worm form a solution containing many worms using a tapered channel and filter. Second, the worm is immobilized for imaging using a flexible PDMS membrane, which compresses the worm against a cover slip. Third, the worm is transported to one of two holding containers. Worm movement through the device is controlled on screen through a custom computer program. This work discusses the design, fabrication and testing results of the microfluidic chips.

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CHAPTER 1

INTRODUCTION

Background

Microfluidics is a growing field formed from the need to analyze small amounts of fluid samples quickly, such as in biology and defense applications. Thus the field primarily focuses on the manipulation and analysis of fluids in channels with micrometer dimensions through the use of valves, pumps, reservoirs, and analysis systems, which include laser and other optical systems. Since channel dimensions are micrometers in scale, only small amounts (pico-microliters) of fluid samples and reagents are needed to perform analysis.

Early microfluidic systems were fabricated of silicon and glass. However, these materials and manufacturing processes are expensive, time consuming, integrate poorly with valves and pumps, and in the case of silicon are not transparent to visible light, thus precluding optical methods of analysis. These manufacturing methods soon gave way to cheaper methods, which used plastics and polymers. The most popular substrate for microfluidic chips is Polydimethylsiloxane (PDMS). PDMS has the advantages of being inexpensive, relatively straightforward and fast to manufacture, and allows for valves and pumps to be directly integrated into the microfluidic chip.

Polymer microfluidic chips have broadened the biological applications of microfluidics. PDMS is transparent, allowing microscopes to be used for analysis. PDMS is also gas permeable, which allows for O_2 and CO_2 to be added or removed from the chips channels and reservoirs easily. The application of microfluidics to solve biological problems has great potential since channel dimensions are on the same order as the dimensions of cells and small organisms. Microfluidics has been applied to such biological challenges as sorting of cells,[1] cell culturing,[2] and sorting of motile and nonmotile sperm.[3] This thesis discusses the application of microfluidics to aid in the study of the Caenorhabditis elegans (*C. elegans*) worm.

Motivation

The *C. elegans* worm has been studied extensively due to its relative simplicity and straightforward use as a model for genetic manipulation. The *C. elegans* worm is one of the simplest organisms that contain a nervous system. The worm also exhibits many desirable traits sought for when studying biological organisms. The worm is transparent, has a life span of about 2-3 weeks with around a 4-day development time, contains many of the same organs as other animals, has a completely sequenced genome, and is relatively inexpensive to maintain.[4] However, due to the worms' small physical size, up to 1 mm in length and ~80 μm in diameter, manipulation of the worm can be difficult.

Microfluidics has been utilized in studying and controlling worms because of its ability to confine the worms within small channels. The worms are able to live in a fluid environment, allowing them to be positioned throughout the chip by flowing fluid

through the channels, which carries the worms with it. A few of the current microfluidic devices that aid in studying the *C. elegans* worm include devices that screen and sort worms based on physical or genetic traits,[5,6] expose worms to stimuli,[7] study learning and behavior of worms using mazes[8] and oxygen gradients,[9] and trap worms[10,11,12] allowing them to be imaged or undergo surgery. The devices of particular interest to this work include those for imaging and screening worms.

Many of the microfluidic devices created to aid the study of the *C. elegans* worm were designed to help with screening of worms. Microfluidic devices have been designed to help with parts of the process and some can perform the entire process. The current methods for screening worms without the use of microfluidics are tedious and time consuming. To image a population of worms they must be immobilized and manually loaded onto a microscope slide. After imaging, any worms of interest must be selected by hand and transferred to a separate container. This screening process becomes a bottle neck when large populations of worms are processed, such as in RNAi or drug screens where thousands of worms need to be imaged and sorted.

Microfluidic devices have been created for high throughput screening of *C. elegans* worms by both Yanik[5] and Lu[6]. Both of these devices and the screening process in general can be broken down into three main steps; separation, immobilization for imaging, and sorting. Several other microfluidic devices have been created to address specific parts of the screening process, and will be covered in their relevant sections below.

The first step in the screening process on a microfluidic chip is to separate a single worm from many. Current nonmicrofluidic methods accomplish this by using a

pick made of a glass pipette with a short flat platinum wire attached at one end. A worm is picked up by hand using the end of the platinum wire and moved to a microscope slide. This technique takes time to perfect and if the screener is not careful can injure the worm. Yanik accomplishes separation by circulating worms through a large channel. A valve on a side channel of the large circulating channel is opened allowing worms to pass into an analysis chamber. A single suction channel too small for an adult worm to pass through located in the analysis chamber is opened pulling a worm against the chamber wall. The remaining worms in the chamber are then flushed out leaving only one worm in the chamber (see Fig. 1). Lu separates a single worm by using a tapered channel, which forces worms to line up in single file. A single worm at the front of the channel then becomes pulled into position by an array of five suction channels much smaller than the worm (see Fig. 2).

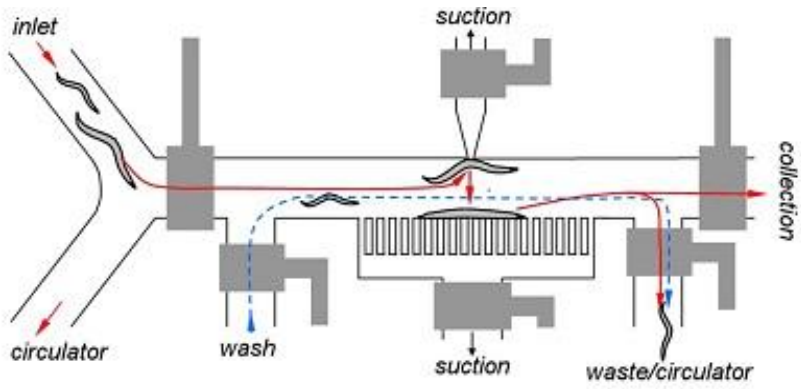

Figure 1: Diagram of Yanik[5] worm screening chip showing separation mechanism. Worms are circulated and brought into the analysis chamber. A small suction channel is turned on pulling one worm against the chamber wall. Remaining worms are flushed out of the chamber leaving only one worm. The separated worm is then immobilized by an array of suction channels.

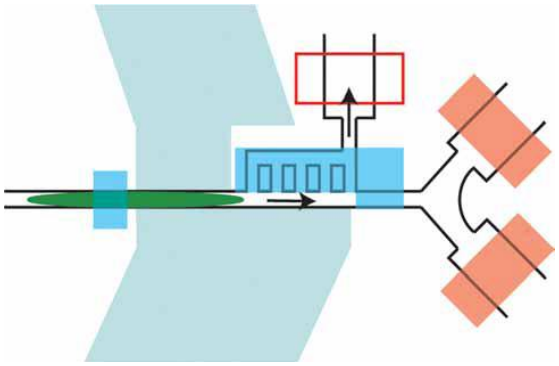

Figure 2: Diagram of separation mechanism in Lu's[6] worm screening chip. Worms are forced to line up in single file and are brought into position by an array of five suction channels too small for the worm to fit through.

Both of these methods rely on the suction channels being smaller than the worm so that it will not pass through them. The chip by Lu also requires the sizes of the worms not vary much, as large worms will not fit in the tapered channel and small worms may travel down the channel side by side.

The next step in the screening process is to immobilize and image the worm. There have been several microfluidic chips designed for this purpose and they offer several advantages over the current immobilization methods. In order to obtain high magnification images or videos of a worm, the worm must be completely immobilized. Nonmicrofluidic immobilization methods include using glue[13] or anesthetics[4] to prepare worms for imaging. Cyanoacrylate adhesive is used to keep worms still but requires a time consuming process performed to each worm individually making it impossible to analyze large populations. Also, gluing of the worms is an irreversible process eliminating further study after imaging. Anesthetics can immobilize large groups of worms quickly; however the effects of these chemicals on the nervous system are mostly unknown.

Microfluidics offers several alternative methods to immobilize worms for imaging, which help overcome the problems associated with anesthetics and glue. Whitesides' group has developed an array of channels that slowly taper and trap several worms at a time with typically only one worm per channel[12] (see Fig. 3).

The chip is designed so that as a worm enters a tapered fluid channel the resistance to fluid flow in the channel increases significantly reducing the flow and causing remaining worms to move towards other unoccupied fluid channels; by this method the chip tends to trap only one worm per channel. This method works well for immobilizing the worms but makes selection of a specific worm after imaging difficult as the only way to remove the worms is to flush them backwards out of the chip.

Another immobilization method uses a thin deformable membrane to compress the worm. A thin flexible membrane is sandwiched between two channels and a worm is brought into one of the channels. The other channel is then pressurized forcing the membrane into the channel with the worm. Both Yanik[10] and Chronis[11] have used this method to image or perform surgery on worms.

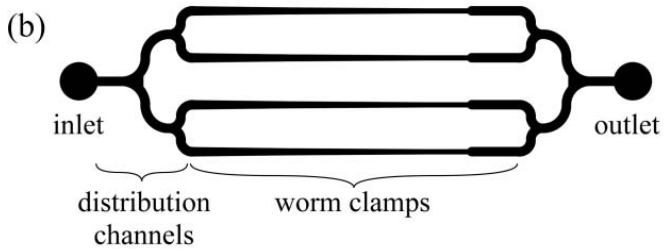

Figure 3: Diagram of the array of tapered channels developed by Whitesides'[12] group. Worms are carried by fluid flow into the channels, which become smaller in diameter than the worms. The worms are restrained by the channel walls thus immobilizing them for imaging.

Yanik was able to compress the worm and take images of the AVM cell body at 50X magnification using the membrane immobilization method and reported some, but little, worm movement in the body of the worm. Membrane immobilization requires little time for activation. The membrane can be moved into place restraining the worm within a few tenths of a second. Chronis reported use of the thin membrane for compressive immobilization but has also used the membrane, which is gas permeable, to fill the channel that contains the worm with CO_2 gas (see Fig. 4). The CO_2 gas acts as an anesthetic and immobilizes the worm within ~1-2 min. The time for immobilization to occur is attributed to diffusion of the CO_2 gas across the membrane.

Worms were successfully immobilized for both short (1-2 min) and long (1-2 hrs) durations using both methods and upon recovery they showed similar locomotion patterns as before immobilization. The CO_2 gas is thought to affect synaptic transmission at the neuromuscular junction and regulatory molecules used in sensing. Therefore CO_2 immobilization would produce undesirable effects if studying the nervous system of the worm.

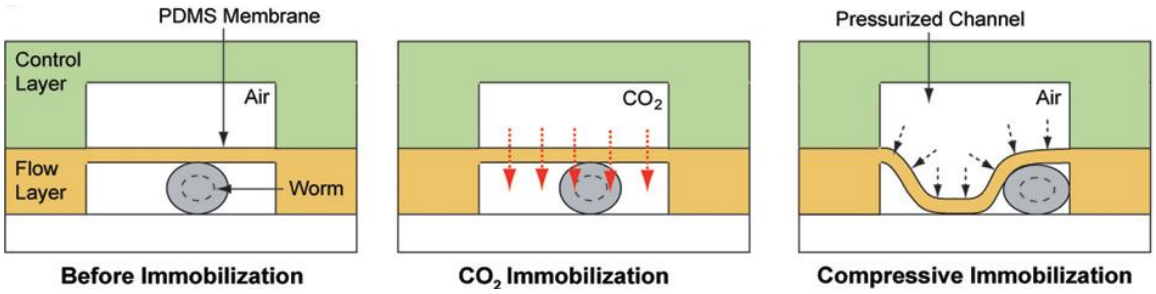

Figure 4: Diagram of Chronis[11] method showing worm immobilization by diffusion of CO_2 across a membrane and immobilization by pressurizing the upper channel forcing a membrane into the channel below compressing the worm.

Also, since immobilization can take minutes, this method would not be feasible for high throughput applications if each worm were to be immobilized individually for a short duration and not as a population.

Other immobilization methods include using small suction channels or cooling. Both Yanik and Lu have created *C. elegans* screening chips based on these methods. By lowering the pressure in an array of channels, which are much smaller than a worm, the worm can be held in a specific orientation and immobilized (see Figs. 1 and 2). The suction channels are operated by external and internal valves and can be activated within hundreds of milliseconds. The suction channel method does hold the worm against the array of channels. However several groups including Yanik's have reported it does not immobilize the worm completely. Cooling of a worm to ~ 4° C has been shown to stop worm movement and allow for imaging. Cooling of worms to near freezing can cause damage or death if held at these temperatures for too long. Since the worm is small in size its temperature can be changed quickly, allowing worms to be immobilized and screened rapidly. Lu's chip, which uses cooling to immobilize the worms, is capable of processing hundreds of worms per hour.

The last step in the screening process is to sort the worms after they are imaged. The microfluidic chips with this capability all use a channel that branches into multiple channels with valves on each branch allowing the output location of the worms to be chosen.

The purpose of this work was to create a set of microfluidic tools similar to those discussed, with some modifications to further the study of the *C. elegans* worm here at the University of Utah. This thesis covers two devices, which were designed for this

purpose. The first device is a muscle force measurement tool used to classify strains of worms based on the contraction force of the main muscles, which run the length of the worm and are used for locomotion. The second device is a worm screening chip designed to immobilize worms for imaging and also provide a means for sorting the worms afterwards. The screening device is intended to reduce the time needed to process worms. This thesis covers the design, manufacturing and testing of these microfluidic tools.

CHAPTER 2

MUSCLE FORCE CHIP DESIGN

Overview

The biology department at the University of Utah has altered wild type worms by knocking out genes that code for specific ligand gated ion channels, which are believed to control muscle contraction. By removing specific ion channels it is thought that the mutant strain of worms will exhibit a difference in muscle contraction force from that of the wild type strain. Due to the small size of the worm, measuring muscle force has proven to be difficult, as conventional methods used on larger organisms cannot be applied. In order to compare the muscle force between the altered strains of worms and the wild type strain, a microfluidic measurement device was created.

The microfluidic muscle force testing chip was based on the two-layer polymer cross-channel technique[14] where the pneumatic control layer and the fluid layer are separated by a thin flexible membrane. As the control layer is pressurized it causes the thin membrane to deflect into the fluid layer (Fig. 4). This concept is used often in microfluidics to create valves and pumps. In this application the deflection of the membrane is used to press against a worm enveloping and immobilizing it. The *C. elegans* worm is found naturally in soil, which it must move through and break into rotting fruit for food. By enveloping the worm with a thin membrane the natural

environment of the worm is mimicked. The pressure against the membrane compressing the worm is slowly increased and precisely controlled using a sensitive air regulator. Our hypothesis is that a correlation can be established between the muscle contraction force of the worm and the pressure acting on the membrane compressing the worm. Comparing the pressures at which different strains of worms become immobilized will allow us to classify the worms according to muscle contraction force.

Design

The muscle force measurement chip consists of three main components: a fluid layer, a flexible membrane, and an air layer (see Fig. 5).

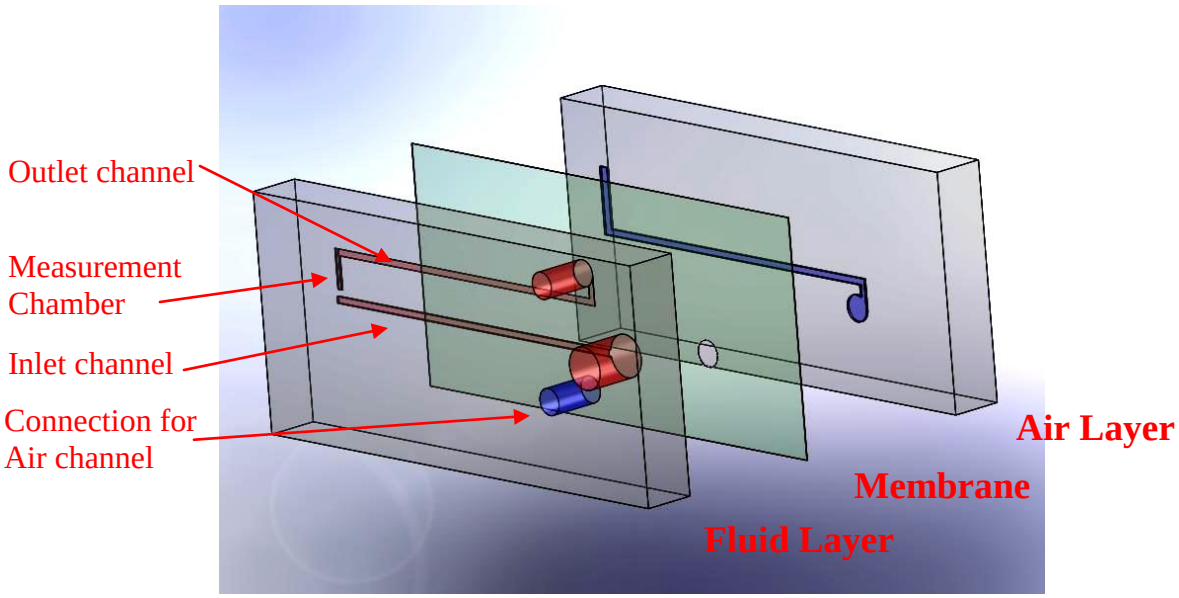

Figure 5: Design of muscle force measurement device showing the fluid, membrane, and air layers.

The fluid layer contains the measurement chamber, inlet, outlet, and a port for connection to the air layer, which passes through both the fluid layer and membrane to reach the air layer. The inlet consist of a channel 500 μm wide by 160 μm tall. The inlet channel leads to the measurement chamber but stops just 500 μm before reaching the measurement chamber. This break between the inlet channel and the measurement chamber is used to create a valve, which keeps the worm from flowing out of the measurement chamber and back into the inlet channel when being compressed by the flexible membrane. The valve works by having the air channel sit directly below the measurement chamber and inlet channel. The valve is opened by pulling a vacuum in the air channel causing the membrane to pull down into the air channel creating an opening between the inlet channel and the measurement chamber. When the air channel is pressurized during testing the membrane then comes back up closing the valve and compressing the worm (see Fig. 6)

The measurement chamber consists of a channel 500 μm wide by 60 μm tall by 3 mm long. The channel steps up in the center another 60 μm with the step being 200 μm wide (see Fig. 7). The purpose of this raised section in the center of the measurement chamber is to maintain the worm centered in the chamber during compression. Without the raised section in the middle of the chamber the worm moves to the edge of the chamber when being compressed. At the edge of the chamber the membrane does not extend easily into the chamber and make good contact with the worm making immobilization difficult. Since the main channel of the measurement chamber is smaller than an adult worm, the worm naturally moves into the stepped section.

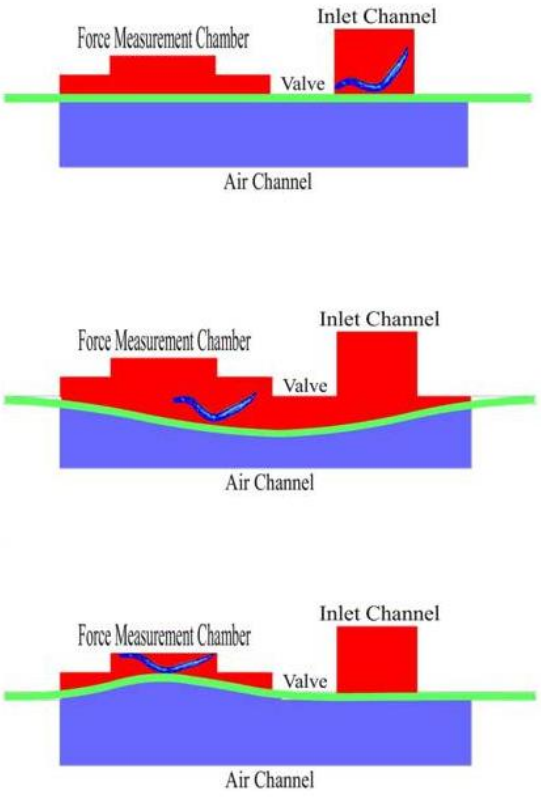

Figure 6: Diagram of muscle force measurement chip operation. The worm is brought down the inlet channel. A vacuum is pulled in the air channel causing the membrane to pull into the air channel opening the valve for the worm to enter the measurement chamber. The air channel is then pressurized extending the membrane into the measurement chamber compressing the worm and also closing the valve preventing the worm from flowing back out of the channel. The outlet channel (not shown) allows fluid to escape the measurement chamber but is too small for the worm to pass through.

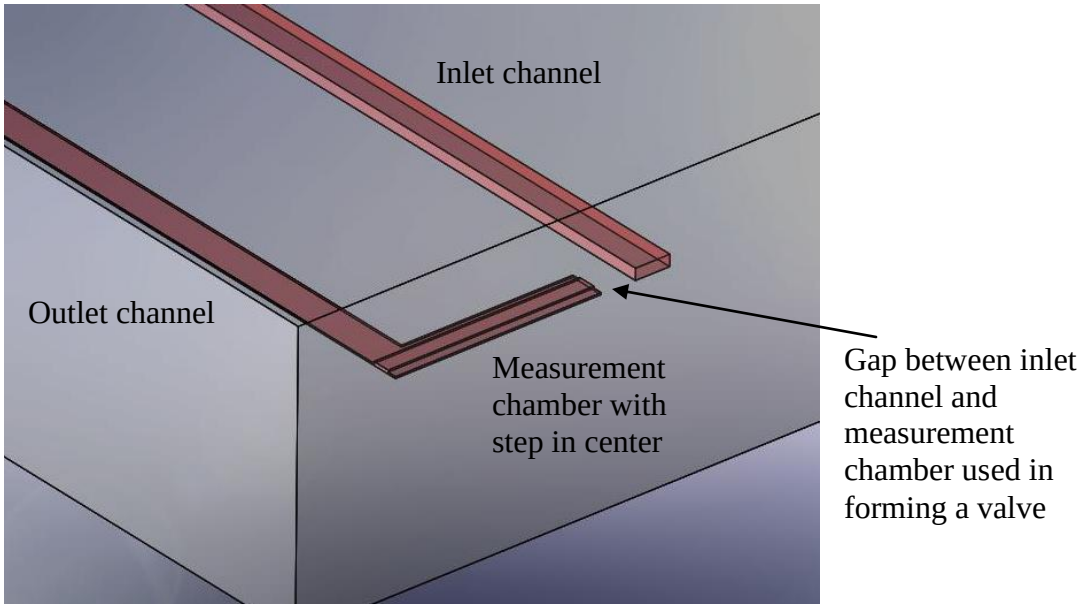

Figure 7: Design of measurement chamber showing stepped section (membrane and air channel below chamber are not shown). Valve between inlet channel and measurement chamber is also shown.

The fluid outlet to the chamber is at the far end of the measurement chamber opposite the inlet. The outlet is 60 μm in height and 500 μm wide. The outlet channel is thin enough that it is difficult for an adult worm to pass through but allows fluid to flow out of the measurement chamber as the membrane extends into the chamber displacing fluid while compressing the worm. The air channel is simply a dead end channel 500 μm wide and 160 μm in height. One end of the channel is connected to the port on the fluid layer and is used for connecting an air supply with regulator while the far end of the channel sits directly below the measurement chamber and inlet channel.

The worms are loaded into the chip one at a time from an agar plate using a worm pick. The worm is loaded into the inlet well and positioned into the measurement chamber by connecting a syringe to the inlet well. Once measurement is complete the worm is removed by either flushing the worm through the outlet channel, which can be

difficult since the outlet channel is smaller than the worm, or moving the syringe to the outlet channel and flushing the worm back through the inlet.

All the layers of the device were designed to be fabricated using PDMS. PDMS was chosen because it is not toxic to the worms, is gas permeable especially through the thin membrane layer allowing oxygen to reach a worm in the chip, and PDMS is also transparent, which allows a worm in the middle of the chip undergoing testing to be viewed using a microscope.

Conclusion

The muscle force measurement chip is a straightforward design utilizing a flexible membrane to compress a worm in the center of the measurement chamber. The flexible membrane is controlled using a sensitive air regulator. Our hypothesis is the pressure at which a worm is immobilized corresponds to the maximum contraction force of the worm's main muscles used for locomotion. The chip has three connections or ports: an inlet, an outlet, and an air connection. The chip requires only one syringe and a regulated air supply to function making it simple to operate.

CHAPTER 3

MUSCLE FORCE CHIP FABRICATION

Overview

The chip was fabricated using xurography techniques in a lab setting without the need for a clean room. Molds were created and a 10:1 ratio of PDMS base to polymerizing agent was poured over them. The PDMS was cured in an oven and once polymerized the layers were aligned and bonded together. The fabrication of the chip is relatively straightforward and fast, typically taking one day to mold and assemble.

Fluid, membrane, and air layers

The chip consists of three layers bonded together: a fluid layer, flexible membrane, and an air layer. The fluid and air layers were made using a xurography technique. The designs for each layer were created in Autocad and cut out of tape using a Graphtec cutting pro FC-5100-75 sign plotter. The tape design was laid out in a petridish creating a mold for PDMS to be poured into. The fluid layer consists of the inlet channel, the measurement chamber, and the outlet channel (see Fig. 5). The inlet channel was cut out of 3M Scotchcal Instachange tape approximately 160 μm thick. In order to make the measurement chamber (including the step) and the outlet channel smaller in height, they were cut out of 3M 200MP double sided tape approximately 60 μm thick. The step in the

measurement chamber was formed by cutting out a piece of the double sided tape 200 μm wide by 2 mm long, which is thinner in width than the main channel in the measurement chamber. This piece cut from the double sided tape was then placed on top and in the center of the main channel in the measurement chamber. The air layer channel design was cut out in the 3M Scotchcal Instachange using the sign plotter and the design was laid out in a petri dish forming a mold for the air layer. The flexible membrane was formed by pouring PDMS onto the bottom side of a petri dish and spinning at 4000 rpm for 1 min. yielding a membrane ~30 μm thick. All PDMS used in fabrication was a 10:1 ratio of base to polymerization agent. All PDMS molds and membranes were cured in a 60° C oven for 3 hrs.

After curing, the PDMS layers were removed from the molds and trimmed. At the inlet and outlet, 1.5 mm diameter holes for syringe connections were cored into the fluid layer. The fluid layer valve was masked off and then bonded to the membrane. To mask the valve the 500 μm gap between the inlet channel and measurement chamber, which is used to form a valve, was covered using Scotchcal Instachange tape. This was done to prevent activation of the PDMS surface below the tape mask so that a bond between the masked area and the membrane would not form when the two layers were brought together. All the PDMS layers were bonded by exposing them to corona discharge, which alters the surface chemistry of the PDMS activating the surface for bonding. An Enercon Dyne-A-Mite 3D Treater corona discharge was used to generate the corona discharge. After bonding the membrane to the fluid layer another 1.5 mm hole was cored through the fluid layer and membrane for the air connection. The air layer was then bonded to the bottom of the membrane layer completing the chip.

Conclusion

Manufacturing of the measurement chip is a fairly straightforward procedure and can be accomplished in roughly a day. Manufacturing does not require special equipment outside the price range of most labs nor did it require use of a clean room facility. The chip design was created in Autocad and molds were formed by cutting the design of each layer from tape and placing the tape in petri dishes. PDMS was poured into the molds and cured forming the individual layers. Access holes to the channels were cored in the fluid layer for loading of worms and connection of a syringe. The layers were then bonded together by activating the surface of the PDMS and bringing the layers together. A multilayered microfluidic chip used for measuring muscle contraction force in the *C. elegans* worm was fabricated.

CHAPTER 4

TESTING AND RESULTS

Overview

The measurement chip was designed to be able to distinguish a difference in muscle force between wild type and mutant worms. The device was tested using three strains of worms: wild type, UNC-29 knockout, and ACR-16 knockout. The *C. elegans* worm contains three ligand-gated ion channels at the neuromuscular junction, which trigger the relaxation or contraction of the muscle cells. These three ion channels are UNC-49, ACR-16, and UNC-29. UNC-49 is known to control relaxation while ACR-16 and UNC-29 are thought to control contraction. However the roles of and relationships between ACR-16 and UNC-29 are not well understood. A worm with UNC-29 knockout has an uncoordinated and slow movement. If ACR-16 knockout is added the worm becomes paralyzed. A worm with only the ACR-16 knockout, however, appears to have normal locomotion. It is thought that UNC-29 controls the main contractive source while ACR-16 is used for further force recruitment. To test whether a worm with ACR-16 knockout has less contractive force than a wild type worm the muscle force measurement chip was used. As a means of validating the device worms containing the UNC-29 knockout were tested and compared against the wild type worms. Worms containing the

UNC-29 knockout move in a slow and uncoordinated way suggesting their muscles have less contraction force than the wild type worms.

Test methods

Nine worms from each of the three strains (wild type, UNC-29 knockout, and ACR-16 knockout) were loaded into the chip one at a time and tested. To test a worm, it was picked from an agar plate and placed into the inlet port, which had been filled with M9 solution.[4] A syringe filled with M9 solution was connected to the inlet port and used to position the worm within the measurement chamber while viewing the chip under a dissecting microscope. In the stepped section of the measurement chamber the worm is able to thrash back and forth. Using a Bellofram model 70 air regulator, pressure in the air channel was slowly increased causing the membrane to extend into the chamber and envelop the worm. Pressure was slowly increased until the body of the worm could no longer thrash side to side. The head and tail were allowed to move but once the body had been restrained and there was no longer thrashing or forward or reverse progress of the worm it was considered immobilized. Restraining of the head and tail of the worm is much more difficult and has proven to require much higher pressures if accomplished at all. When the worm became immobilized the pressure in the air channel was recorded. The worm was then flushed from the chip using a syringe filled with M9 solution and another worm was loaded. This procedure was followed until all 27 worms had been tested. Fig. 8 shows the testing setup of the chip with regulator.

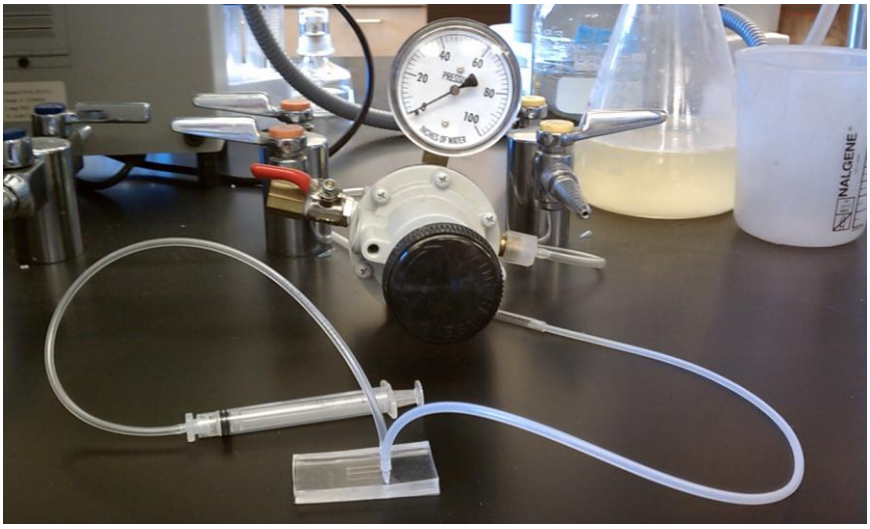

Fig. 8: Testing setup of muscle force measurement chip. The regulator is used to pressurize the air chamber, and the syringe is for positioning worms.

Results

The mean pressure required for worm body immobilization for each strain along with the standard deviation is shown in Fig. 9. The immobilization pressures ranged from 30 to 80 in. H_2O. The results from testing the three worm strains in the device indicate a difference in the muscle contraction force between worm strains. The pressure measurements show the worms with the UNC-29 knockout have a lower mean immobilization pressure at 37.11 in. H_2O than the wild type strain at 50.44 in. H_2O. This was expected and agrees with the visual results, which show that a worm with UNC-29 knockout is uncoordinated and slow moving. Worms with the ACR-16 knockout could not be visually distinguished from the wild type worms by their movement and therefore needed to be tested using the device. By using the force measurement device the difference could be seen between the ACR-16 knockout and the wild type strains.

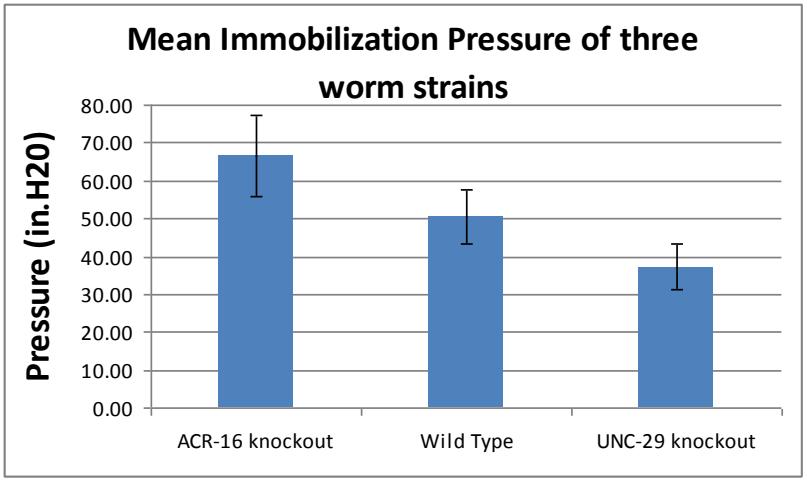

Figure 9: Mean immobilization pressures and standard deviations of three worm strains. ACR-16 knockout, Wild type, and UNC-29 knockout

When the ACR-16 knockout worm strain was tested the worms showed an increase in pressure required for immobilization over the wild type strain. Worms with the ACR-16 knockout had a mean immobilization pressure of 66.67 in. H_2O. This result of ACR-16 knockout worms requiring a higher pressure to become immobilized than that of the wild type worms was not expected. The hypothesis was that the worms with the ACR-16 knockout would fall somewhere between the wild type worms and worms with the UNC-29 knockout. This report does not attempt to explain the reasoning for this result and focuses more specifically on the functionality of the device.

A T-test was performed to determine if statistical differences existed between the mean immobilization pressures of the UNC-29 knockout and the wild type, and between the ACR-16 knockout and the wild type. Both tests returned p values less than $1X10^{-3}$ indicating a significant difference in immobilization pressures and that the device is capable of ranking worms based on immobilization pressures, which we believe corresponds to muscle contraction force.

Conclusion

Three strains of worms were tested in the microfluidic device to determine its ability to rank the muscle contraction force of the *C. elegans* worm. In total 27 worms were tested in the device and comparisons of the results indicate that the device is capable of ranking the worms according to muscle contraction force based on the pressure required for immobilization. Measurements taken by two means showing similar results help in validation of the device. When UNC-29 knockout was viewed optically it was apparent that it was slow and uncoordinated having less muscle force than the wild type worms. The same result was achieved when measured in our chip, which showed UNC-29 knockout as requiring much less pressure to immobilize.

CHAPTER 5

WORM SCREENING CHIP DESIGN

Overview

The techniques and principles learned by creating the muscle force measurement chip were used to create a microfluidic worm screening chip. The screening chip was designed to decrease the time needed to screen worms based on images and video of fluorescently labeled worms. The original design of the screening chip was patterned after the screening chip produced by Lu[5] with some minor modifications. A replica of the Lu chip with a modification to the immobilization technique was created as a starting point. The Lu chip uses cooling to immobilize the worms while our chip uses the membrane immobilization technique. However, the initial prototype based on the Lu design did not function well at separating or immobilizing the worms so several modifications were made to the original design and are discussed in detail in this chapter.

The final chip design can be broken down into the same three steps as was done for the screening chips discussed previously. These are separation, immobilization, and sorting. Screening of worms is a serial process so the worms must be separated out and imaged individually. Separation is performed by moving the worms through a channel, which forces them to line up single file and then selecting only the worm at the front. The single worm is immobilized for imaging using a thin membrane technique similar to

that in the muscle force measurement chip discussed previously. After imaging the worm is sent to one of two outputs and collected thus sorting the worms.

The chip was designed to be used on a Zeiss Axiovert 200M confocal microscope at up to 100x magnification with an oil immersion lens. External dimensions of the microfluidic chip were designed so the chip would fit on the stage of the microscope and tubing connections were designed to not interfere with the microscope operation. A glass cover slip was attached to the bottom of the chip to interface the chip with the microscope lens. Several prototypes of the entire chip and of specific sections were tested throughout the design process contributing to the final chip design. The final chip design and manufacturing are discussed in the following sections.

Design specifications

In order to establish a guideline of the necessary functions and design constraints of the screening chip a specifications document was created. The following is a list of the general design specifications. These specifications are discussed in further detail in the design section.

1. Load worms grown on an agar plate into the chip, or if worms are grown in solution the worms must be loaded from the solution.
2. Separate one worm from a population.
3. Immobilize a single worm without damaging so that images and video may be taken at 100x magnification.
4. Sort worms into two separate containers based on user input.

5. The device must be compatible with the Zeiss Axiovert 200M confocal microscope.
6. Control of the worms in the chip is to be performed by the user via computer program with intent of full automation in future.

Design

The entire chip other than the cover slip attached to the bottom was designed to be made of PDMS because of its compatibility with the worms. PDMS is oxygen permeable through thin membranes and is not toxic to the worms. PDMS is also transparent making it ideal for use on the microscope.

The chip consists of three main layers: an air layer used for control of valves and pressurization of the immobilization membrane, a separation and sorting layer, and an immobilization layer (see Fig.10). A cover slip is attached at the very bottom of the chip for imaging purposes and also forms the bottom of the immobilization area. Two thin flexible membranes were used, one between the air and separation / sorting layer used to form valves, and one between the immobilization layer and the cover slip used to immobilize the worm. The chip also has a thin layer with a channel cut out of it between the immobilization membrane and the cover slip. This thin layer with channel creates a filter for positioning the worms within the immobilization area. These layers and their functions are discussed in detail below.

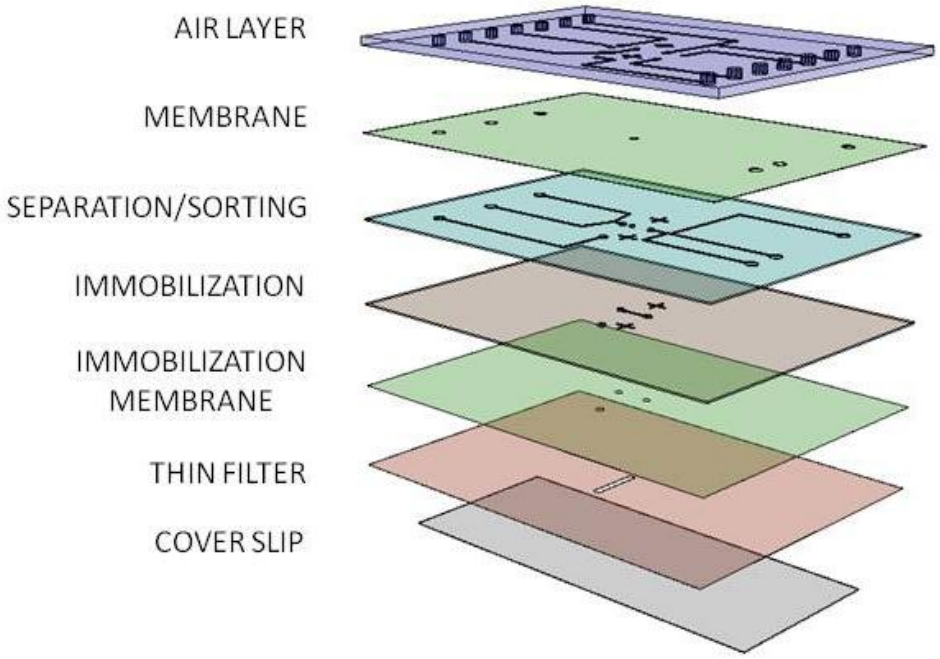

Figure 10: Autocad drawing showing the position of each layer in the screening chip.

As previously mentioned the chip can be broken down into three main areas: separation, immobilization and sorting. The Autocad drawing of these areas on the chip is shown in Fig. 11. The design explanation of the chip is split into these three areas.

Seven on chip valves, which control the movement of worms and fluid throughout the chip, are pneumatic seat or gap valves.[15] The open channel valves[14] were tried in the beginning but did not close well or stop fluid flow, especially with the higher aspect ratio channels in the separation area of the chip.

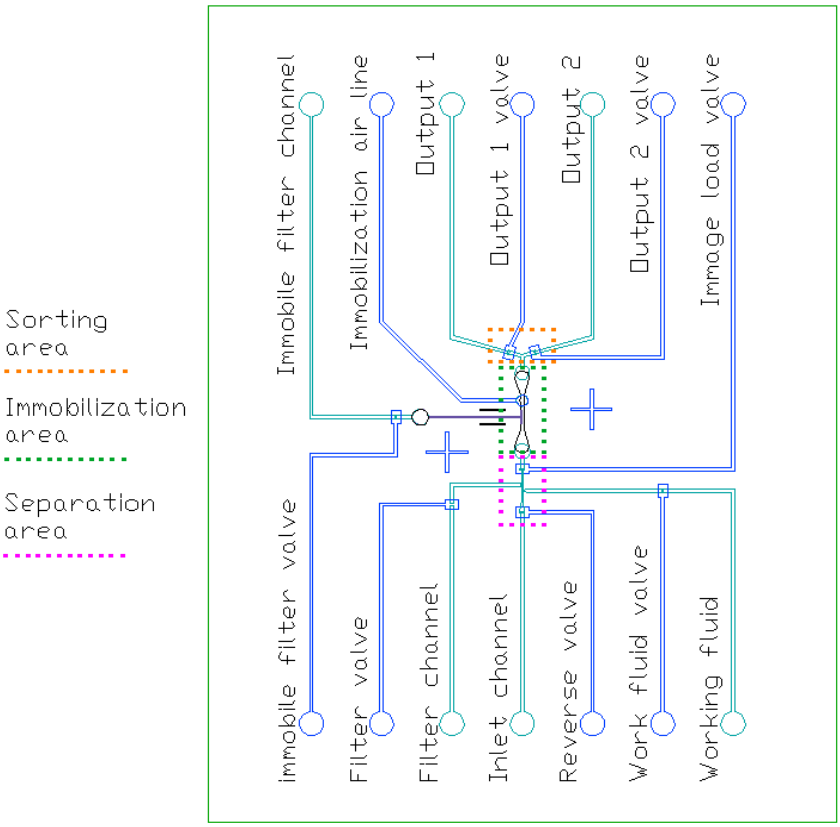

Figure 11: Autocad drawing of chip design showing three main areas of chip: separation, immobilization, and sorting. Input ports for fluid (green) and air (blue) connections are shown.

Loading of worms

Worms are loaded into the screening chip by a pressure driven fluid flow, which carries the worms with it. A Gene-Mate 50-1250 µl pipette tip was found to be an ideal container to generate the pressure driven flow. The pipette tip is used to rinse worms to one side of an agar plate and then suck up the worm containing solution. The tip of the pipette is then inserted into the inlet port of the chip. Since the chip is made of PDMS it creates a tight seal around the pipette tip when inserted into the inlet port. A PDMS plug with attached pressure line is then inserted into the top of the pipette tip sealing the top allowing it to be pressurized.

Separation of a single worm

Separation of the worms is accomplished by pressurizing the pipette tip with the worm solution in it moving the worms down the inlet channel. The inlet channel tapers from 200 μm wide to 80 μm wide forcing the worms to line up single file in the narrowed section. The depth of all the channels in the separation section is approximately 80 μm. The worms move single file down the inlet channel to an array of 4 small channels 12 μm wide with a spacing of 50 μm between them. The four small channels intersect the inlet channel perpendicular to it. These small channels are too small for young adult worms to fit through and act as a filter allowing fluid to pass through but stop the worms. When the worms reach the filter channels, the pressure in the pipette tip containing the worm solution is released and a working fluid channel is turned on. The working fluid channel intersects the inlet channel 240 μm up stream of the filter channels, roughly at the tail of the front worm. When the working fluid valve is opened the fluid flow holds the front worm against the filter channels while the rest of the worms are pushed backwards down the inlet channel. The reverse valve is then closed leaving only one worm in the separation area. The working fluid channel has a small opening 35 μm wide at the intersection of the inlet channel. The small opening helps to impede worms from entering as they move past it towards the filter channels. The working fluid is contained in a pipette tip with the same set up as that which contains the worm solution. The working fluid pipette tip is always under pressure and fluid flow is controlled by the on chip working fluid valve. Fig. 12 shows the operation of the separation mechanism.

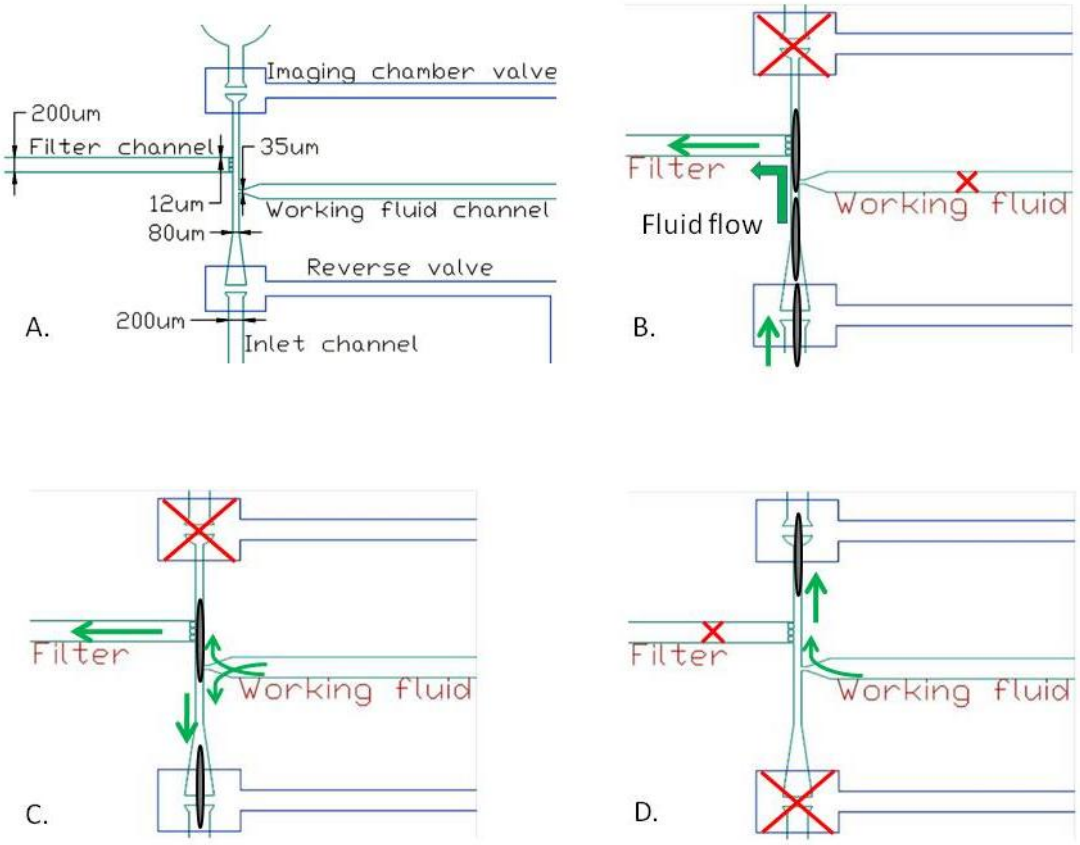

Figure 12: Diagram showing operation of separation mechanism. a) Autocad drawing of separation area with dimensions. Figures b-d show a sequence of separation mechanism with fluid flows. b) Worms are brought up to filter in single file by pressurizing fluid in pipette tip. c) Pressure in pipette tip is vented and working fluid valve is turned on pushing front worm against filter and remaining worm back down inlet channel. d) Reverse valve and filter valve are closed. Imaging chamber valve is opened. Working fluid pushes single worm into immobilization area.

Several iterations were made of the separation section of the chip to find working dimensions for the filter and the tapered channel, which forces worms to line up single file. One of the biggest challenges was designing a system that can handle variations in worm size. The tapered section if made too small causes larger worms to become stuck clogging the system. Too large a tapered channel allows for smaller worms to travel side by side. A channel approximately 80 μm wide by 80 μm high was the best compromise fitting both large and small young adult worms. After a worm has been separated, the imaging load valve is opened and the working fluid pushes the worm into the imaging area.

Immobilization and imaging

The imaging area consists of a channel 200 μm wide with flared ends for coring vias, which allow the worm to move between the separation / sorting layer and the immobilization layer. The Autocad drawing of the immobilization area is shown in Fig. 13.

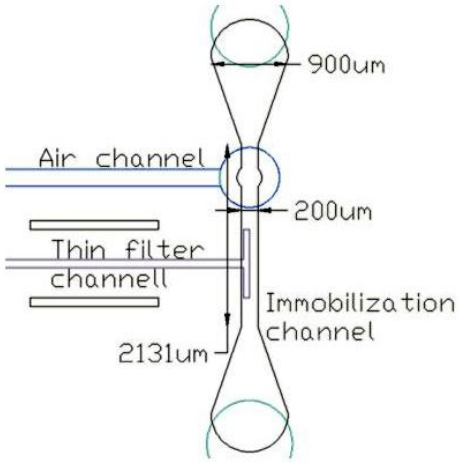

Fig. 13: Autocad drawing of immobilization area of chip with flared ends.

There are two purposes for having the imaging area on a separate layer than the other chip functions. First, the working focal distance of the confocal microscope at 100x magnification using an Olympus Tirf 1.49 NA lens is small at approximately 250 μm. The confocal microscope lens sits against a cover slip attached to the bottom of the chip. By having the worm drop down a layer the worm is able to sit directly against the cover slip, as the cover slip forms the bottom of the imaging area.

The second purpose for having the worm drop down a layer is the immobilization method. In the traditional method a flexible membrane extends down into the channel containing the worm pressing against it. This method tends to push the worm to the side of the channel where the membrane does not extend easily nor conform well to the worm's body. It is especially difficult to restrain the head and tail of the worm, which taper to a smaller size than the body. The traditional method works well for low magnification (10X) imaging since the worms do not appear to be moving much, but at high magnification (100X) it is evident the worms are still moving significantly, especially near the head and tail. By having the worms drop down a layer to the cover slip, the orientation of the setup is changed so that the flexible membrane rests flat on the cover slip with the immobilization channel above it. Pulling a vacuum in the channel above the membrane causes the membrane to pull away from the cover slip and into the channel above creating a space between the membrane and cover slip. The worm drops down a layer through the via and into this space. The thought is that if the membrane typically rests flat where the worm is being immobilized, it will conform much better to the worm especially near the head and tail of the worm, which have been difficult to

restrain in previous versions of the chip using the traditional membrane restraint design. A comparison of the two methods is shown in Fig. 14.

Several thicknesses of the immobilization membrane were tested ranging from 2 μm to 40 μm with the worms being restrained better with the thinner membranes. It is believed this is due to the thinner membranes conforming more easily to the worm's body. The final chip design used a 2 μm thick membrane to immobilize the worms.

Having the worm drop down through the immobilization layer and the thin immobilization membrane to the cover slip requires a special design of the immobilization channel. The immobilization channel is flared out at the ends and must be rounded. During manufacturing, when the thin immobilization membrane is attached to the immobilization layer it is pressed in at the flared ends of the channel, which creates a space at each end of the channel for a hole to be cored through the layers forming vias for the worm to pass through while maintaining a sealed chamber above the membrane (see Fig. 15).

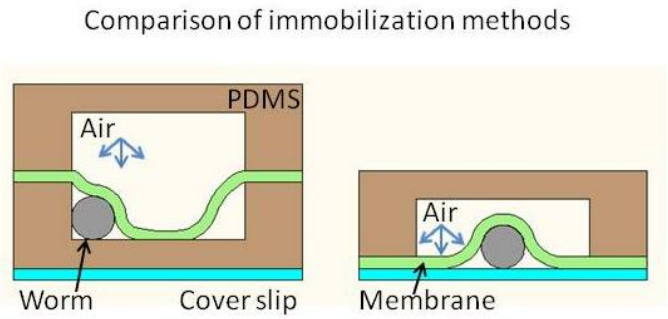

Fig. 14: Comparison of two membrane immobilization methods. The traditional method, shown at left in the figure, extends the membrane into a channel, which contains the worm, compressing it. The alternative method applies negative pressure in the air chamber causing the membrane to pull away from the cover slip creating a space between the membrane and the cover slip, which the worm is moved into. A positive pressure is then applied to the air chamber compressing the worm against the cover slip.

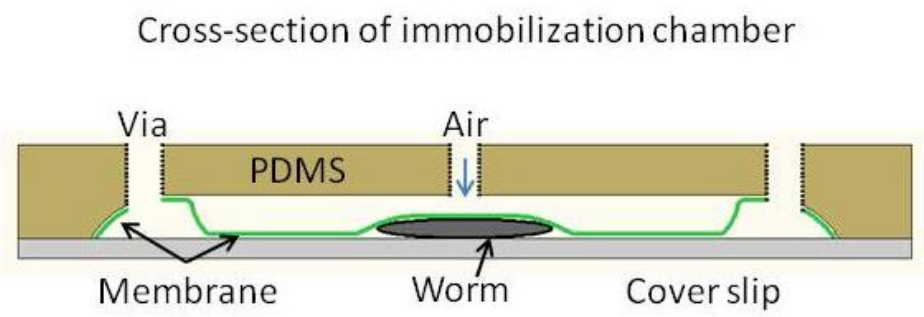

Fig. 15: Cross-section of the immobilization channel design showing membrane pressed into channel at ends, which is done during bonding of the membrane to the layer. Vias are cored at channel ends for worms to move between layers.

Worms were moved into the immobilization area by two methods. In the first method, the immobilization membrane was opened and closed quickly (1 sec pulses). This moves the worm along in increments into the immobilization area. In the second method a thin ~15 μm thick layer of PDMS with a channel cut from it is put between the cover slip and immobilization membrane during manufacturing. The cut out channel in this layer is placed directly under the immobilization area and acts as a filter, which allows fluid to pass but is too small for a worm to pass through. This method is meant to position the worm in nearly the same spot every time reducing the amount the microscope stage must be moved to bring the worm into view.

Although the design and manufacturing would be simpler if the worm did not have to move between the separation / sorting and immobilization layers, the design of the separation method and gap valves used throughout the chip prevent a single layer design.

Sorting

After the worm has been immobilized and imaged it is brought back up a layer to the separation and sorting layer. Sorting is accomplished by splitting the main channel, which is 200 μm wide into two channels each 200 μm wide at a Y junction (see Fig. 16). A valve is placed on each channel near the Y junction with the valves as close to the junction as possible to help prevent worms from entering the wrong channel. Opening one valve while closing the other directs fluid flow and worms down the correct channel. Corning 2 ml round bottom cryogenic vials with barb connections glued to the bottom are inserted at the outlet ports of the two Y junction channels for collecting the worms. This design could be expanded to accommodate more than two channels if necessary.

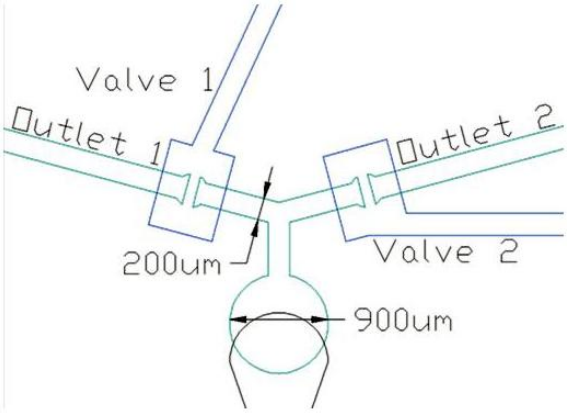

Fig. 16: Autocad drawing of sorting area on chip. Sorting is accomplished by having the main channel split at a Y junction. Valves are placed near the junction on each branch controlling worm output.

Conclusion

The screening chip was designed to incorporate the necessary functions to fulfill the design specifications laid out previously. The functions of the chip, which fulfill the design criteria, are listed below.

1. Worms are loaded into the chip from an agar plate using a pipette tip.
2. A single worm is separated from the population using a tapered channel and small channels, which act as a filter for the worms.
3. The separated worm is immobilized for imaging using a thin flexible PDMS membrane, which compresses the worm against a cover slip.
4. After imaging, the worm is sent to one of the two outputs and collected for further use by having the worm travel down either the left or right channel at a Y junction.
5. The external chip dimensions were designed so it would fit on the stage of the confocal microscope for imaging and a cover slip was used to interface the chip with the microscope lens.
6. The on chip pneumatic valves, which control fluid and worm movement through the chip, are controlled using a computer program and could be set up for full automation in the future. The valves and software are discussed more fully in the controls / automation section.

CHAPTER 6

WORM SCREENING CHIP FABRICATION

Overview

The screening chip was manufactured almost entirely out of PDMS using soft lithography techniques. The chip was designed using Autocad and molds for each layer were made in the University of Utah Nanofab. PDMS was poured into the molds and cured forming blocks with channel designs imprinted. The thin membranes used were made by spinning PDMS onto silicon wafers. The PDMS layers were cut to fit on the stage of the confocal microscope, aligned, and assembled in layers along with the thin membranes to form the complete chip. A cover slip was bonded to the bottom of the chip to form the bottom of the immobilization area and to interface with the microscope lens. Bonding of the layers and of the cover slip was achieved by exposing the PDMS and glass to oxygen plasma activating their surfaces. Masking of the layers before bonding was achieved using a water soluble mask.

Fabrication of molds

Molds were made of the three main layers: air, separation / sorting, and immobilization. In order for the worms to fit in the channels the mold structures needed to be ~80 μm thick. To form such thick structures SU-8 is preferred to positive resist as

most positive resists are suited for much thinner layers. The air layer and the separation / sorting layers were made using SU-8 and yielded structures 80 μm in height using the following procedure.

1. Dispense SU-8 2050 on wafer and spin at 2,500 rpm for 30 sec.
2. Bake SU-8 for 2 min. at 65° C followed by 5 min. At 95° C.
3. Expose at 7mJ / cm^2 sec. for 13 sec.
4. Post exposure bake 1 min. at 65° C followed by 5 min. at 95° C.
5. Develop using SU-8 developer.

The Immobilization layer was made using AZ 9260 positive resist, which was reflowed to form a rounded structure. SU-8 is not able to reflow and so cannot be used to make rounded channels. The design of the immobilization channel, which allows the worms to drop down a layer to the cover slip, is such that the ends of the channel need to be round. The immobilization membrane conforms and bonds to the round channel ends when pushed in during bonding of the two layers. The AZ 9260 resist is meant to form thick structures but was still spun on in three layers in order to create channel structures approximately 80 μm tall. Several attempts were made until a procedure was found to create tall structures. The following procedure yielded structures 75 μm tall.

1. Dispense room temperature photoresist to cover 4 in. wafer, spin 30 sec. at 2000 rpm. This applies a thin layer of photoresist on the wafer and helps reduce the photoresist from peeling off the wafer.
2. Bake 30 sec. at 110° C.
3. Dispense photoresist to cover wafer, spin 30 sec. at 800 rpm.

4. Rest at room temperature for 5 min. This step allows solvent to slowly evaporate preventing bubbles from forming.
5. Bake resist 1 min. at 110 ° C.
6. Dispense photoresist to cover wafer, spin 30 sec. at 800 rpm.
7. Rest resist at room temperature for 5 min.
8. Bake resist 5 min at 110° C.
9. Expose resist to UV light at 7mJ / cm^2 sec. for 12 sec. on, 30 sec. off, repeated 11 times.
10. Develop in 3:1 water: AZ400K.
11. Reflow resist 2 min. at 130° C. to form rounded structures.

After fabrication the molds were treated to prevent adhesion of PDMS to the structures. The molds were placed in a vacuum chamber with 30 μl of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane and left under vacuum for 1 hr.[16]

Fabrication of PDMS layers from molds

All layers were made using a 10:1 ratio PDMS to curing agent. The PDMS was degassed and poured into the molds. The air layer was poured thicker than the rest at approximately 5 mm so that the PDMS on this layer would be thick enough to connect barb fittings for air and fluid lines. To reduce the height of the vias the worm must move up and down through, and to help with alignment of the layers, the separation / sorting layer and immobilization layer were made as thin as possible. The separation / sorting layer was poured to just cover the structures with the resulting PDMS layer approximately 1 mm thick. Thinner layers were attempted but upon removing the PDMS

from the SU-8 mold the PDMS would tear. The immobilization layer mold was reflowed and had smooth structures so it could be made thinner. The immobilization layer was formed by spinning PDMS on the wafer mold at 300 rpm for 30 sec. All the layers were cured on a hot plate at 75° C. for 30 min.

The membrane layer, which attaches to the air layer and is used for on chip valves, was made by spinning PDMS on the bottom side of a petri dish at 3,000 rpm for 30 sec. The very thin membrane used for immobilization was fabricated following the technique developed by Glucksberg's group.[17] A 10:1 mixture of PDMS was diluted by adding 10% by weight Hexane. The mixture was then poured on a smooth wafer with no structures, which had been coated with Cytop to prevent adhesion. The wafer was spun at 6,000 rpm for 60 sec. and baked on a hot plate at 75° C for 30 min. The thin membrane was removed from the wafer using the following the procedure. Uncured PDMS was spread on a circular PDMS ring whose inside diameter is slightly smaller than the outside diameter of the silicon wafer. The PDMS ring was then laid on top of the thin membrane at the edge of the wafer and cured on a 75° C hot plate for 30 min. After curing, the circular PDMS ring was lifted from the wafer pulling up the membrane also and holding it suspended in the middle of the ring.

The thin filter layer, ~15 μm thick between the cover slip and the immobilization layer, which forms a filter for positioning the worm within the immobilization area, was made by spinning a 10:1 mixture of PDMS on a Cytop coated wafer at 6,000 rpm for 60 sec. The PDMS was cured on a 75° C hot plate for 30 min. The thin layer was removed from the wafer using the circular PDMS ring method discussed above. The thin layer was laid on a plate of glass and a CO_2 laser was used to cut a channel out of the PDMS.

A laser was used to cut out the channel because molding such a thin layer with a section removed proved to be impossible as the PDMS would tear when attempting to remove it from the mold.

Assembly of layers

All the layers were bonded using an Enercon Dyne-A-Mite 3D Treater corona discharge altering the surface chemistry from containing methyl groups to hydroxide groups. When two treated layers are brought together they create a bond through a condensation reaction. This technique eliminates the need for glues or liquid PDMS bonding, which if not applied carefully, can wick into channels clogging them. In certain areas of the layers bonding is not desired as is the case with valves and the immobilization membrane. To prevent bonding in these areas a mask is applied blocking the surface from being activated. Tape can be used as a mask but requires the desired shape be cut out, applied to the PDMS before activation and then removed after activation before bringing the two layers together. This process can be even more difficult when the areas to be prevented from bonding are small requiring placement and removal of the tape while viewing under a microscope.

A process was created for masking areas, which does not require removal after activation of the surface before bonding and is easily removed once the chip is assembled. To mask an area, a thin layer of liquid soap is applied to the PDMS surface with the tip of a fine pair of tweezers. A template can be used to guide application if necessary. The soap blocks the oxygen plasma preventing bonding. Once the chip is assembled the

masking soap is removed by flushing the channels with water. The soap forms a good mask since it covers the hydrophobic PDMS evenly but is easily removed after assembly.

The layers were assembled in a specific order so the vias between layers could be cored and to facilitate alignment of the layers. The specific sequence of assembly follows. See Fig. 10 for break-down of layers.

1. The immobilization layer is bonded to the separation / sorting layer (back to back) no masking is required.
2. Using a modified 27 ga. needle a hole is cored through the two layers in the middle of the immobilization chamber for air to pressurize the immobilization membrane.
3. The thin immobilization membrane is bonded to the immobilization layer and the membrane is pressed into the channel at each end causing the membrane to bond to there.
4. Using a 0.75 mm coring tool, a via is cored at each end of the immobilization channel where the membrane was pressed in. The vias allow the worms to pass between layers.
5. All the air ports on the air layer are cored using a 1.5 mm coring tool.
6. The membrane that was spun on the petri dish is bonded to the air layer and the fluid ports on the air layer are cored using a 1.5 mm coring tool.
7. The valves on the separation / sorting layer are masked to prevent bonding and the separation / sorting layer is bonded to the air layer.
8. The immobilization membrane is masked off in the area over the immobilization channel and then bonded to the thin filter layer.

9. A Fisher Scientific 22x50-1 cover slip is then bonded to the thin filter layer.

Conclusion

The sorting chip was manufactured by fabricating molds in the University of Utah Nanofab. The molds were then used to create PDMS blocks with structures imprinted. These blocks along with PDMS membranes were aligned and assembled in layers to form the channels and valves on the screening chip. A new process was created for masking the layers before being exposed to oxygen plasma and bonded. By using the soft lithography technique it was possible to create a multilayered chip with integrated valves and internal vias between layers.

CHAPTER 7

CONTROLS / AUTOMATION

Overview

The screening chip has internal pneumatic valves for controlling fluid and worm movement on chip. External solenoid valves are used to control air flow to the on chip pneumatic valves. By activating an external solenoid valve a corresponding internal pneumatic valve is activated. An external solenoid valve is also used to pressurize and vent the pipette tip containing the worms. The solenoid valves are connected to a 24 channel I/O board connected to a computer via USB and interfaces with a customized Labview program. Pressure gauges are used to control the air pressure for the internal valves and for the pressurizing of the pipette tips (see Fig. 17).

Hardware

The external solenoid valves controlling the air pressure running to the on chip pneumatic valves are three way SMC Pneumatics S070 solenoid valves. Both a pressurized line and vacuum line are connected to the solenoid valves. When the solenoid valve is switched on, pressure is sent to the on chip pneumatic valve closing it. When the solenoid valve is switched off, a vacuum is pulled at the on chip pneumatic valve causing it to open.

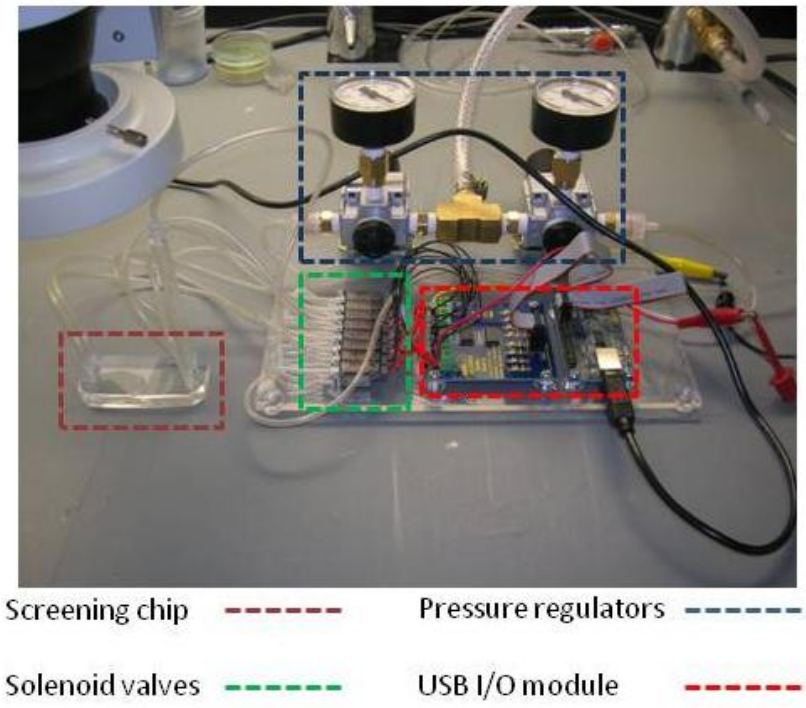

Fig. 17: Picture of controls module and screening chip. The I/O module is used to control the solenoid valves, which control the on-chip pneumatic valves. Pressure gauges are used to regulate the pressure to the valves and pipette tips.

Six solenoid valves were used to control the seven on chip valves (The filter valve and reverse valve were coupled together). One solenoid valve was used to control the pressurization membrane and another for pressurization of the pipette with worm solution for a total of eight solenoid valves used. The S070 solenoid valves were used because of their compact size and low power requirement.

The solenoid valves were connected to the computer through an Elexol USB I/O 24 R Digital I/O Module. An Elexol 8x Darlington Transistor Output Board was also used in conjunction with the I/O module since the solenoids required slightly more current than the USB I/O module could handle.

Two Marsh Bellofram M1 0-30 Psi pressure gauges were used to control the pressure for the on-chip valves and the pressure for the pipette tips. The-on chip valves were operated at pressures between 10-15 psi while the pipette tips were operated at pressures between 1-3 psi. The pressure gauges and other hardware discussed was mounted on an acrylic sheet as shown in Fig. 17.

Cole Palmer 1/16" C-FLEX tubing and Value Plastics 1/16" polypropylene barb fittings were used for all connections between the solenoid valves, pressure gauges, and screening chip.

Software

The computer program used to control the solenoid valves was custom designed using Labview software. The program has six on screen buttons corresponding to preset valve configurations that perform the different functions on the chip. When a button is selected, the solenoid valves are activated and an on screen image of the chip indicates the active valves. The six buttons with valve configurations are:

1. SEPARATE – This setting pressurizes the pipette tip with worm solution bringing the worms into the chip and lines them up single file to the separation filter.
2. REVERSE – The working fluid is turned on and the pressure in the pipette tip with worm solution is vented. The working fluid pushes the front worm against the filter while the rest are forced back down the inlet channel leaving only one worm in the separation area.
3. LOAD – The single worm is moved into the immobilization area for imaging.

4. IMAGE – The immobilization channel is pressurized forcing the immobilization membrane against the worm immobilizing it for imaging.
5. SAVE – The worm is released and moved down the save outlet channel into a collection vial.
6. DISCARD - The worm is released and moved down the discard outlet channel into a collection vial.

The control system is set up so that each step in the screening process is manually controlled on screen but could be easily changed to perform some or all of the functions automatically.

Conclusion

The movement of the worm through the chip is controlled by on chip pneumatic valves, which in turn are controlled by external solenoid valves. The on chip valves turn on and off pressure driven fluid flows carrying the worm throughout the chip. The external solenoid valves are controlled by selecting buttons in a customized computer program. The program was designed so that in the future it could be set for a timed sequence allowing for full automation.

CHAPTER 8

TESTING AND RESULTS

Overview

The manufactured chip and controls were tested to evaluate whether they met the design specifications. The computer controls were tested to make sure all the external solenoid valves and computer program were functioning as desired. Worms were then run through the chip while viewing under a dissecting microscope to test the separation and sorting functions. After refining of pressures and timing of valves to get the separation and sorting functions working properly, the chip was placed on the confocal microscope to evaluate the immobilization function and ability to image worms in the chip.

Test methods

The same strain of worms was used throughout all testing of the screening chip. akIs 141 worms were chosen because of their fluorescent tagging. The akIs 141 strain contains a deletion in the endogenous glr-1 gene. An array containing the wild type gene for glr-1 tagged with GFP has been inserted on chromosome II. The fluorescent tagging can then be used to follow the expression of glr-1 in the neuron AVA. To prepare a plate of worms for testing several adult worms were transferred to a fresh agar plate. The plate

was left for approximately 3-4 days at 20° C. yielding a plate containing mostly young adults.

Testing of the manufacturing process and computer controls was performed by selecting each setting in the computer program to verify the correct solenoid valves were activated. The test was performed while running water through the chip to check for properly functioning on chip valves.

The ability of the chip to separate one worm from a population and the ability to sort worms by sending them to multiple outputs was tested. Worms of the akIs 141strain were loaded into a pipette tip by rinsing them to one side of an agar plate containing mostly young adult worms with approximately 300 µl of M9 solution and then sucking up the solution. The pipette tip was then inserted into the inlet port on the chip. The pressure to the on chip valves was set to 10-15 psi and pressure to the pipette tips was set between 30-100 in H_2O (~1-3 psi). The working fluid pipette tip was filled with M9 solution. The custom lab view program was used to run the chip through the separation, immobilization, and sorting steps while being viewed under a dissecting microscope. A USB camera was attached to the microscope and videos were taken of the testing process.

The ability of the chip to immobilize worms for fluorescent imaging was tested on a Zeiss Axiovert 200M confocal microscope. Worms were loaded by the same method as in separation and sorting testing above. During the immobilization step, Metamorph software was used to take images and video of the worms at 10x, 40x, and 100x magnification to evaluate the ability to view worms in the chip and effectiveness of worm immobilization. Fig. 18 shows the screening chip on the stage of the confocal microscope.

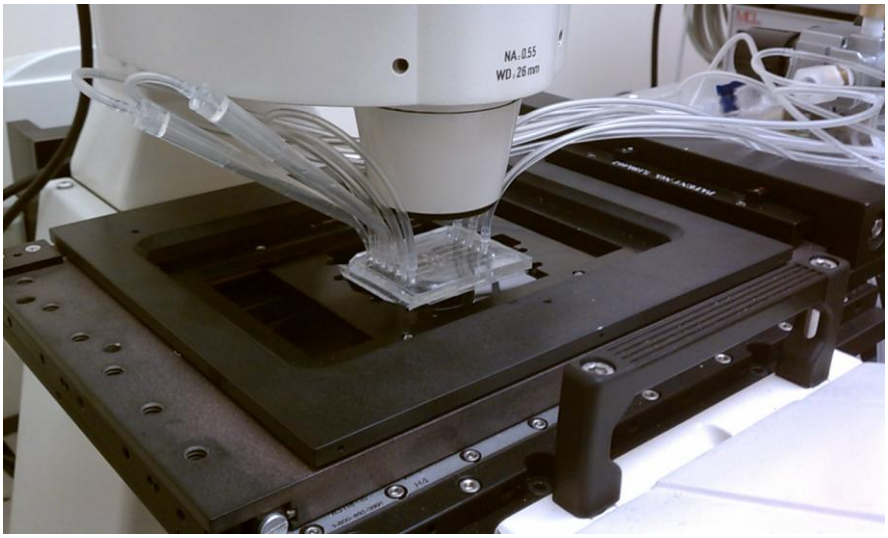

Fig. 18: Screening chip positioned on the stage of the confocal microscope.

Results

Initial testing of the custom Labview program and manufacturing process showed the solenoid valves activated correctly according to on-screen selections and the on-chip valves functioned properly.

Loading of the worms was found to be simple. Loading the worms onto the chip was fairly straight forward and could be done in less than a minute by rinsing the worms on an agar plate and sucking the solution into a pipette tip, which was inserted into the inlet channel.

Testing of the separation function with young adult worms showed good success once the pressure in the pipette had been adjusted. The worms lined up in single file and the separation process worked well with worms being separated typically in less than 2 seconds. Once the pressure in the pipette with worm solution was dialed in for a given run the separation mechanism worked well. Fig. 19 shows photos of the separation sequence from video taken during operation.

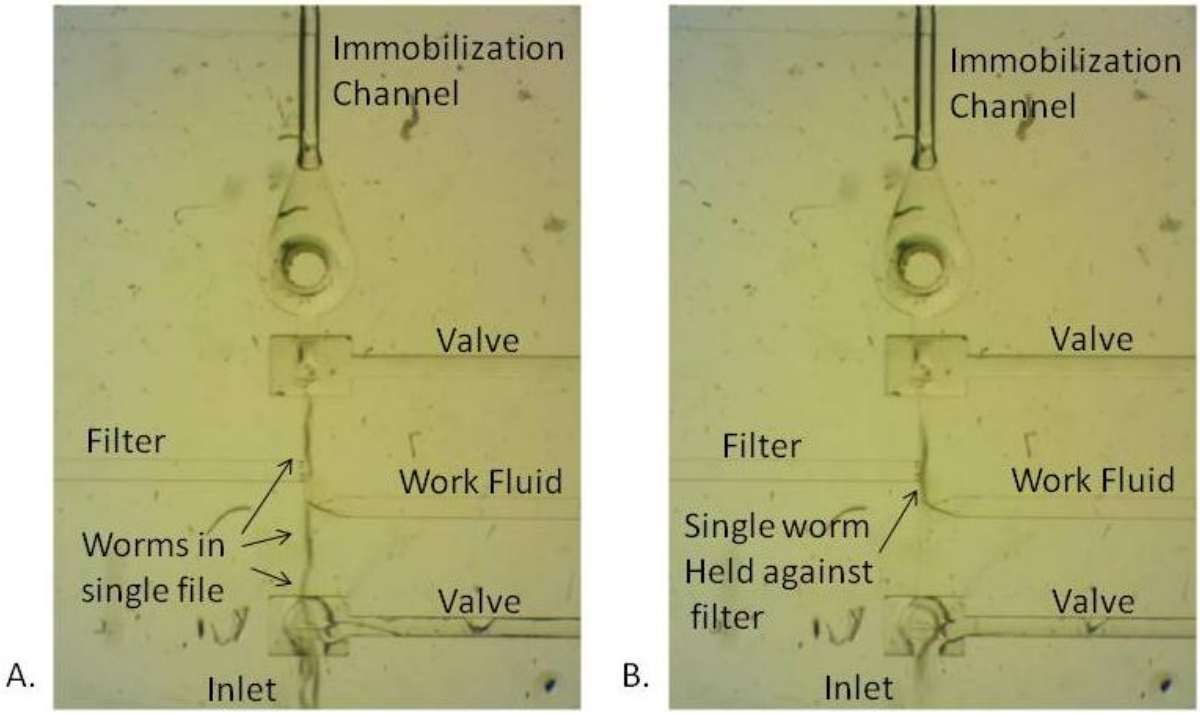

Fig. 19: Photos showing the separation process. A) Worms can be seen lined up in single file up to the filter channel. B) A single worm has been separated and is held against the filter.

The pressure of the pipette with worm solution is critical for the separation mechanism to function properly. Too high a pressure was found to push worms together instead of lining them up in single file in the channel. Too high a pressure also caused some of the worms to flow through the filter channels. Running too low a pressure did not generate enough flow in the channel to carry the worm quickly to the separation area. A balance was found at a pressure of 60 in H_2O (~2 psi). Variation in worm size did have some effect on the separation process. Worms, smaller in size than young adults, caused the separation process to fail more often. The smaller worms were able to fit through the filter channels and at times caused two worms to be separated instead of one. Synchronization of worm growth could prevent this from occurring.

The sorting function of the chip worked nearly perfectly during testing. Throughout all the testing performed only a couple worms moved out of the fluid flow and entered the wrong channel during sorting. Although the worm had entered the wrong channel the valve in the channel was closed preventing the worm from going to the wrong output. In each case the worm would swim back into the fluid flow and be sorted correctly. Although this is not a concern at this point, this could become an issue if the device was fully automated and the sorting function was on a timed sequence.

Testing on the confocal microscope showed that the worm was able to be imaged in the chip while on the microscope. The immobilization function of the device was refined several times throughout the design process and became better with each design. The final prototype immobilization method was able to hold the body of the worm immobilized well enough to be imaged at high magnification. However, the worm was still able to move its head and tail preventing video of the worms near these areas. Images could be taken, but it was difficult to image a specific area with the worm moving. Figs. 20 and 21 show images taken of worms in the screening chip. Fluorescent imaging and video at 100x magnification of the pharynx near the posterior bulb, which is the region of interest in the worms tested, was not able to be taken due to the worm moving in and out of focus. The membrane immobilization of this prototype with the new membrane configuration was better than previous methods. However, the method still needs to be refined in order for high magnification video of the head of the worm.

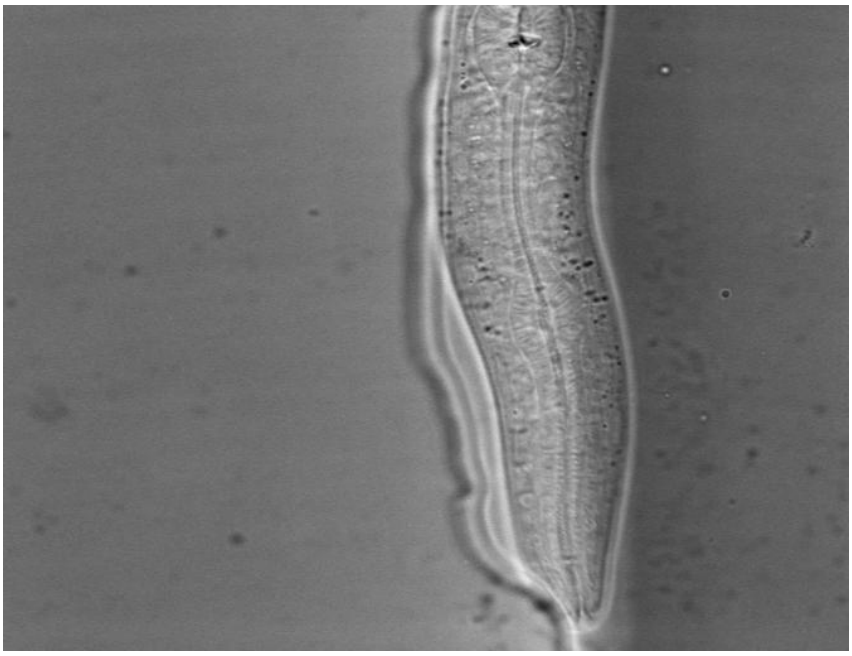

Fig. 20: Photo of the head of a worm at 40x magnification. Photo was taken on the confocal microscope using the screening chip.

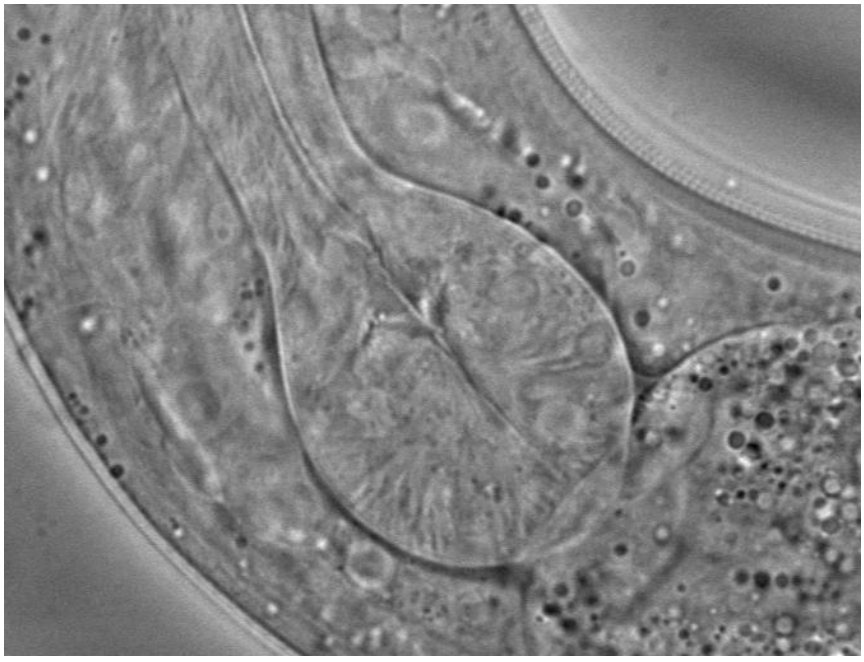

Fig. 21: Photo of the posterior bulb in the pharynx of a worm at 100x magnification. Photo was taken on the confocal microscope using the screening chip.

Conclusion

The chip was tested against the design specifications and testing showed positive results for the prototype as only one area fell short of the specifications. Initial testing of the chip was conducted. However the immobilization section is not performing as well as desired and a project deadline did not allow for further design or testing to be conducted.

The separation and sorting functions were tested by viewing the chip under a dissecting scope. The immobilization function was evaluated by viewing the worms using a confocal microscope. The sorting function of the chip worked nearly flawlessly with only a few worms out of all tested entering the wrong channel. The separation function of the chip worked well with the majority of the worms being separated properly. Occasionally two worms would be separated instead of one or a smaller worm might pass through the filter.

The immobilization function on the chip was designed, tested, and redesigned several times in an attempt to completely immobilize the worms. The initial prototype of the immobilization function slowed the worm significantly, but did not completely immobilize it. The immobilization method of the final prototype was able to restrain the body of the worm, but the worm was still able to move its head and tail slightly. Near the head of the worm in the AVA neuron is the area of interest in the akIs 141 worm strain and movement of the head while in the screening chip was enough that video could not be taken. Yanik[10] in reporting on membrane immobilization also saw some movement of the worm at 50x magnification while looking at the AVM cell body and axon. The proposal for this device was that we could improve on the membrane immobilization

method, which would allow us to look near the head of the worm at high magnification. The membrane immobilization method has been improved upon and with further development the chip would be useful for high magnification screening. The chip as it stands has shown that it may work well for lower magnification (50x) screening of worms or where the area of interest is in the body of the worm, which can be fully restrained.

CHAPTER 9

CONCLUSION

Summary

The application of microfluidics to aid in the study of the *C. elegans* worm has shown promising results. In this work two devices were created for the study of the *C. elegans* worm. The first device was designed to classify three strains of the *C. elegans* worm according to contraction force of the main muscles used in locomotion. This was accomplished by creating an environment similar to the natural environment of the worm, which became more and more restrictive. The device uses a flexible membrane to compress a worm in a channel immobilizing it. Air pressure on one side of the membrane causing the membrane to compress the worm is slowly increased until the worm can no longer move forward or back. Using this method the air pressure can be correlated to the muscle contraction force of the worm. By comparing the immobilization pressures of several worm strains the worms can be ranked according to muscle force. Three strains of worms were tested and a difference in the muscle contraction force of each strain was shown. The wild type worms had a mean immobilization pressure of 50.44 in. H_2O. Worms with the UNC-29 knockout worms had an immobilization pressure of 37.11 in. H_2O, which was less than the wild type and was expected as this agreed with visual results. The ACR-16 knockout had an

immobilization pressure of 66.67 in. H_2O, which is higher than the wild type worms and was not expected, as the ACR-16 ion channel was thought to be used for further force recruitment in muscle contraction. Further study of the worm and use of the device may help to explain this result. The microfluidic chip created contributes to the study of the *C.elegans* worm by helping solve the problem of measuring muscle force.

The second device created to help research of the *C. elegans* worm was a microfluidic chip designed to decrease the time needed to screen worms from images and video taken on a confocal microscope. Similar devices had been created by others and the screening chip was designed so researchers at the University of Utah would have access to these tools. The screening chip functions by pulling a worm from solution, immobilizing the worm for images and video to be taken, and then sending the worm to one of two holding containers for further study. Design specifications were laid out and upon testing of the device it was shown that all the specifications were achieved but one. Several conclusions have been made from testing of the design.

The separation mechanism used in the chip worked well and was capable of separating one worm from the population upon finding the appropriate pressure settings and timing of valves. Some improvements to channel geometry and placement of the working fluid channel may increase performance.

The sorting mechanism of the chip worked near flawlessly and was capable of delivering the worms to the correct holding container.

The chip was capable of moving worms between layers and a method was created for holding the worms directly against a cover slip for imaging.

The immobilization mechanism of the chip could not fulfill the design specifications as it was not able to completely immobilize the head and tail of the worm. Further design of the immobilization method may produce a membrane based method, which is capable of immobilizing the head and tail of a worm.

The chip designed could be useful for screening worms where complete immobilization of the head and tail is not necessary. Although video requires the worm to be completely still for a significant amount of time, screening based only on images of the worm may be possible as the worm does not move quickly while under immobilization.

Future work

Both of the devices built for studying the *C. elegans* worm would benefit from further work. Future work on the force measurement chip would include further testing with a larger number of worms. The use of a thinner more compliant membrane may help to distinguish the force between strains even more.

The worm screening device still needs work to become a fully functional tool for screening the *C. elegans* worm. The area in need of the most improvement is the immobilization method. The current method restrains the worm but not quite enough for high magnification imaging and video. Some further design and testing has been performed and suggests that by placing a thin layer of 30:1 PDMS base to curing agent between the cover slip and the immobilization membrane the worm can be completely restrained. The hypothesis is that the worm is able to move while being compressed by the membrane due to the low friction coefficient between the worm and the cover slip.

The friction coefficient is increased by spinning a thin layer of PDMS on the cover slip. It would also be useful to look into using other immobilization methods such as cooling of the worm.

The current prototype design was based on testing of individual components which were combined to form a final device. This contributes to the need for the worm to move between multiple layers. Future work should include combining the layers into one single layer, which would reduce the manufacturing time and may allow the separation area to be combined with the imaging area.

Further work on the input and output of worms would be beneficial for RNAi screening where strains of worms could be pulled from a 96 well plate screened and then output to a 96 well plate.

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