BACHELOR

Making gel layers with defined height using microfluidic

van Venrooij, Jeroen I.W.

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EINDHOVEN UNIVERSITY OF TECHNOLOGY

Department of Mechanical Engineering
Microsystems

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Bachelor End Project

J.I.W. van Venrooij

Supervisor:
Dr. R. Luttge

Lab coach:
G. Akçay

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Department of Mechanical Engineering
Microsystems

Eindhoven University of Technology
Building 15, Gemini-Noord (Floor -1)
de Zaale, 5612 AJ Eindhoven
The Netherlands
www.tue.nl

Author
J.L.W. van Venrooij (1023436)

Supervisor
Dr. R. Luttge

Lab coach
G. Akçay

Date
3rd February 2023

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J.L.W. van Venrooij (1023436)
j.l.w.v.venrooij@student.tue.nl

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1 Abstract

In brain-on-chip research, important factors for understanding brain function are the structural connections and cell–cell interactions in brain tissue. Most of the existing setups are manufactured by using a membrane as a physical barrier in order to make a three dimensional structure of two stacked layers of tissue. There is a desire for devices that can make these tissue layers without a membrane in between their interface. This project explores the possibilities of making this setup by making a device that can make layers of hydrogel with a defined height, so that a membrane is no longer needed. A polydimethylsiloxane (PDMS) mold was made that could make this defined height by making a grooved channel to pin layers at the desired height. Later in the project, nutrient channels will be added next to the existing grooved channel to be able to stimulate the cells locally. The project succeeded in manufacturing a PDMS mold that can pin two layers of hydrogel, although filling the main channel and nutrient channel without them mixing is still a challenge.

2 Introduction

With today's medical research we have gained a large amount of knowledge about diseases, their symptoms and how to treat them. However, for many diseases the underlying mechanism of their onset and progression is still largely unknown [1]. More research is needed to understand these mechanisms to be able to treat and cure diseases more effectively. The medical research field of organ-on-chip focuses on simulating functions and processes that take place in the human body, in order to gain insight of how they work and how we can manipulate them for our own benefit. This project is part of brain-on-chip research, which is on of the organ-on-chip fields. In brain-on-chip research the majority of experiments are done *in vitro* (in glass, in a petri dish). A typical research setup can be seen in Figure 2.1 [2], which is part of a blood-brain barrier experiment. For this project it is not important what this setup is used for. What is important, is the structure within the red circle. Here, a structure can be seen consisting of two tissues with a perforated membrane in between. The tissue in red is blood vessel tissue, in blue is brain tissue. The membrane is used as a physical barrier to make stacked layers of tissue. This is a common method to manufacture three dimensional structures [3].

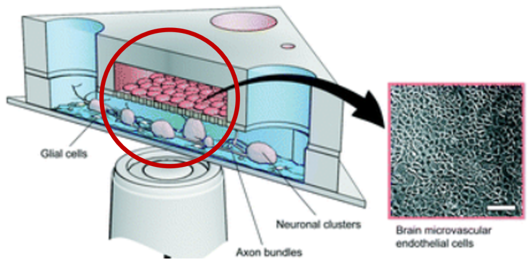


Figure 2.1: Example of a blood-brain barrier setup

For brain tissue, structural connections and cell–cell interactions are important factors for brain function [1]. Different parts of the brain have different structures with different material properties. A biocompatible material that is often used to simulate brain tissue is hydrogel. Hydrogel can be made with different material properties, to simulate the different brain structures. The interface between different tissues is on of the main interests in the research. There is a desire for devices that can make a structure of stacked layers of hydrogel without a membrane in between, to be able to research their interface. In order to make these layers without a membrane you need to be able to make layers with a defined height. This project explores the possibilities of making a device that can make layers of hydrogel with a defined height, so that a membrane is no longer needed.

3 Aim of the project

3.1 Microfluidics: capillary action

As mentioned in chapter 2, the aim for the project is to make a device that can make layers with a defined height. A phenomena from microfluidics called capillary action will be used to make the defined height. Capillary action is the result of interaction forces between a solid and a liquid [4]. On the one hand there are cohesive forces. Cohesive forces attract molecules of the same material. On the other hand there are adhesive forces, which attract molecules from a different material. In Figure 3.1 is a reservoir with blue liquid and three vertical tubes with different diameters. The cohesive forces try to keep the liquid together, while the adhesive forces between the liquid and the tube wall attract each other. When the diameter of the tube is relatively small, the surface area of the tube wall becomes large compared to the volume of liquid within the tube. The adhesive forces will be stronger than the cohesive forces. As a result, the adhesive forces will pull the liquid upwards in the tube to a level higher than in the reservoir. The smaller the diameter of the tube, the stronger the adhesive force will get compared to the cohesive forces, and the higher the level of the liquid will rise. This is why in this project the tubes and channels in the design will be kept as small as possible, to make the best use of the capillary action. As can be seen in Figure 3.1, the liquid at the top in the tube has a curved surface. This is called a meniscus. It is the result of adhesive forces at the tube wall pulling the liquid upwards, while at the same time the cohesive force in the middle of the tube pull the liquid slightly downwards since the adhesive forces become smaller as you go further away from the tube wall.

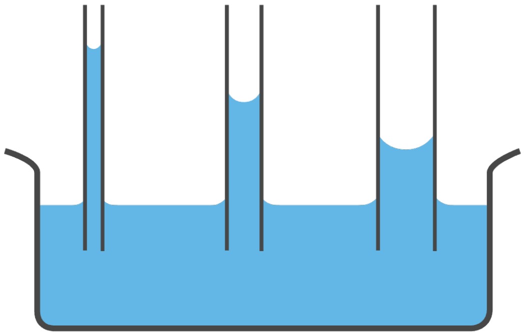
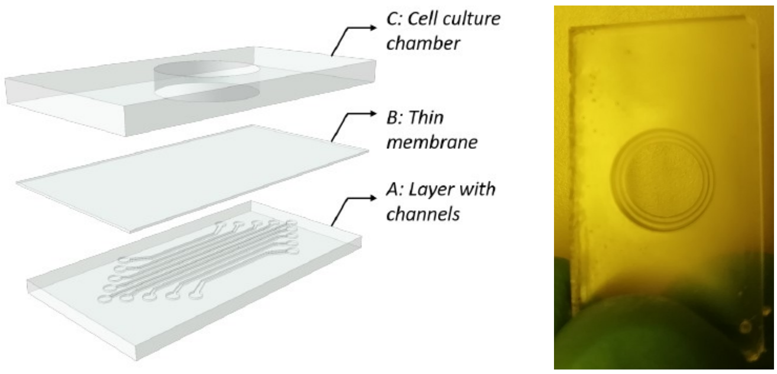


Figure 3.1: Capillary action: adhesive forces are stronger than the cohesive forces, pulling the liquid upwards in the tube

3.2 The actuator

This project builds upon the work done in the bachelor end project of Emre Deniz [5]. In his project, an actuator was made consisting out of 3 layers (see Figure 3.2a). The bottom layer (A) has grooves in them that will function as air channels. In this project it will be referred to as the air channel layer. The middle layer (B) is a thin layer with a thickness of $10\mu m$ that functions as a membrane. This is the membrane layer. The top layer (C) is a reservoir that can hold the cell culture used in experiments. This is the reservoir layer. The air channels are connected to a machine that can regulate the air pressure through the channels. By changing the pressure you can vibrate the membrane on top of it. This allows the organic material in the reservoir layer to be stimulated by vibration.



(a) (b)

Figure 3.2: The 3-layer actuator used by Deniz, with on the right a top view of the PDMS cylindrical reservoir layer

At first, the reservoir layer was just a cylindrical hole, so no layers could be made. A second design was made stacking a larger cylinder on top of a smaller cylinder, creating two layers (Figure 3.2b). This was the first attempt to pin a layer of hydrogel. It did not work to make two layers of hydrogel.

The plan for this project is to make a device where its shape makes use of capillary action to pin hydrogel at a desired height, to make the defined height. This layer will be a replacement for the current reservoir layer from the actuator from Deniz. Figure 3.3 shows an ideal end result for the project: a reservoir layer with a grooved channel (red arrow) that pins both layers of hydrogel (blue) to make two stacked layers of hydrogel.

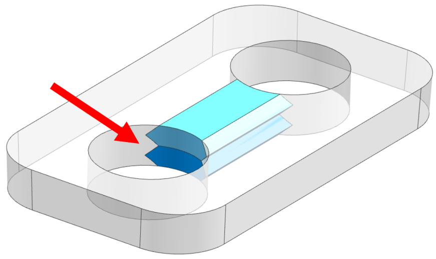


Figure 3.3: Ideal case: 2 stacked layers of hydrogel (blue) in a PDMS mold

3.3 The material

Each layer from the actuator from Deniz is made out of a material called polydimethylsiloxane (PDMS) using molds. For more information about making PDMS and making the layers, see Appendix A. PDMS is an elastomeric polymer with excellent properties for chip fabrication, including biocompatibility, gas permeability, good mechanical properties, optical transparency and simple fabrication by replica molding [6].
To make PDMS a material called SYLGARD^{TM} 184 Silicone Elastomer is used, which consists of two components: a base and a curing agent. The ratio of these components affects the material properties of the PDMS. The air channel layer and the reservoir layer are both made out of PDMS with a mass ratio of 7:1 (base : curing agent), while the membrane layer has a mass ratio of 10:1. The 10:1 ratio makes the membrane a bit more flexible, which is beneficial for a membrane.
Although there are more biocompatible materials available apart form PDMS, this project will not look for alternatives. The reservoir layer from Deniz has a PDMS mass ratio of 7:1, this will be used in this project as well.

3.4 Tools for fabrication

Figure 3.4: First mold, made with an extrusion printer

To make a PDMS part as in Figure 3.3 a mold is needed. Before the start of this project, a first mold was made out of polylactide (PLA) using a extrusion 3D printer (see Figure 3.4). It has two vertical cylinders with a grooved channel in between them. The grooved channel will be referred to as the main channel, as more channels will be added later on. The mold is filled via the cylinders, the grooves of the main channel will pin the hydrogel to make the defined layers. Due to the larger area of the cylinders, more volume is needed to fill the channel. This will slow down the rise of the level of the liquid during the filling, which gives a more controlled input flow.
At first inspection the surfaces of the print are not that smooth. The print resolution of this printer is not sufficient for the size at which this design will be printed. It will negatively affect the quality of the main channel. It will become worse when the size of the dimensions of the groove will be scaled down in the future. An alternative method is needed to make a mold with more detail. That is why it was decided to switch to a different 3D printer: the Formlabs Form 3 Stereolithography (SLA) printer (Figure 3.5).

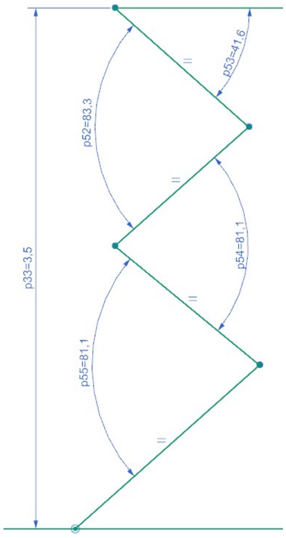
An SLA printer is a printer that builds models out of layers where for each layer an UV laser is solidifying liquid resin [7]. The printing process consists of 3 steps. The first step (left machine in Figure 3.5) is the printing itself as just described. The models in this project will be made out Clear V4 resin. This resin has a high resolution and has optical transparency, which makes it easier to observe what happens in the mold during filling. The second step (middle machine) is an ultrasonic bath with isopropanol (IPA) to rinse of the printed model. The last step (right machine) is UV curing of the model. While

Figure 3.5: The Formlabs Form 3 SLA printing process

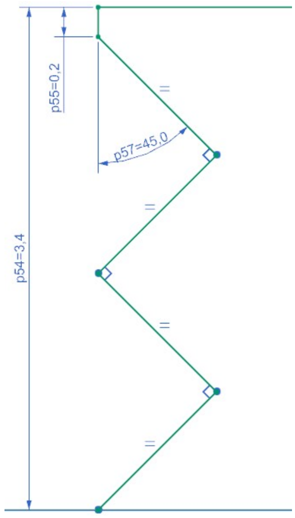
being exposed to heat as well as UV light the resin will
start crosslinking, which hardens out the material and improves its material properties. With its $25\mu m$ layer height (when using Clear V4 resin) [8] and $25\mu m$ XY resolution (horizontal plane) [9] it can print with a higher quality than a extrusion printer.

3.5 Model for the mold

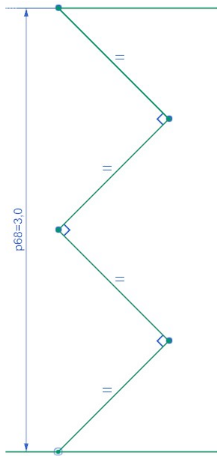
At the start of the project it was unclear what the dimensions were for the mold from Figure 3.4. There were two CAD models that were slightly different from each other. No dimensions could be retrieved directly from these models. To find the dimensions, the models had to be replicated in the CAD software Siemens NX by tracing their outlines. Figure 3.6a and Figure 3.6b show the dimensions of the main channel. Since the main channel is symmetric, only half of the feature is shown. No documentation was available to explain the shape and size of the main channel. Dimensions were chosen to make a base model, with a profile of 90° (45° on top and bottom) angles and a total height of 3mm (Figure 3.6c). The focus will be on improving this design, so not completely redesigning it.



(a)



(b)



(c)

Figure 3.6: Features (a) and (b) come from, (c) is chosen as the base feature for this project

4 Results & Discussion

This chapter consists of two parts. The first part discusses the fabrication of the resin mold and the PDMS part. The second parts discusses the experiments with filling the PDMS mold to make the desired layers.

4.1 Making the PDMS mold

4.1.1 Levelling the mold

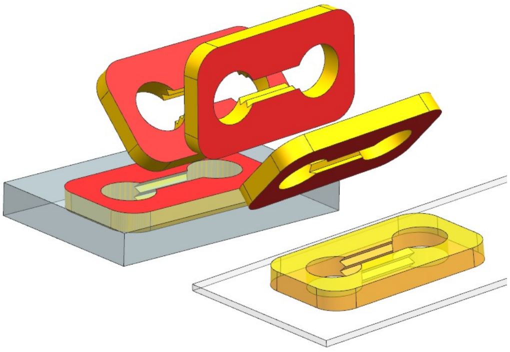


Figure 4.1: The (red) top surface of the PDMS that was on top in the grey mold will end on the bottom when it is bonded to a glass microscopic slide

A first test filling the original mold from Figure 3.4 showed that the top surface of the PDMS did not come out flat. The top surface not being flat is a problem. Experiments showed that any printed surface is not smooth enough to be bonded to another PDMS part, not even with the highest printing resolution. This means that the PDMS surface that was touching the resin mold cannot be bonded. The top surface (red in Figure 4.1) did not touch the grey mold and is the only surface that can be bonded, so the PDMS part needs to be flipped upside down to bond it to other parts. When the top surface can also not be used because it is not flat than the PDMS piece cannot be bonded at all.
The mold can make a PDMS part that looks like Figure 4.2 when the PDMS is filled to the same height as the top side of the main channel. When we look at the left side of this PDMS part and cut it at the pink plane, we end up with the cross section from Figure 4.3. Here it can be seen that a meniscus forms (blue dotted lines), especially around the edges of the part and around the cylindrical holes.

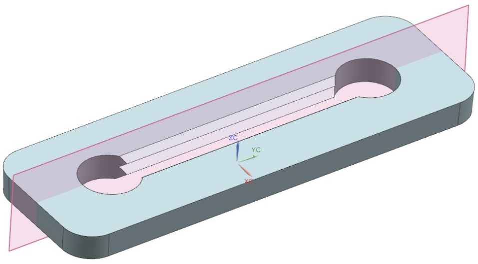


Figure 4.2: CAD model of the PDMS part after filling the mold from Figure 3.4

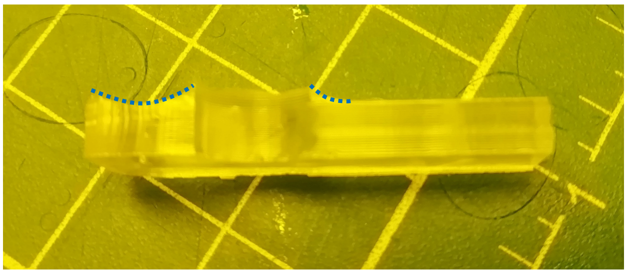


Figure 4.3: Cross section of PDMS

In this mold the level of the cylinders and the outer edge was higher than that of the main channel. A new mold where all three parts have the same height showed that levelling the mold helped with getting rid of the meniscus. However, it was still a slow and precise task that required patience. Figure 4.4 is a schematic cross section of a simple blue mold that is filled up to three different levels. For bottom red line the level of the PDMS is lower than the height of the edge of the mold. The interaction forces between the PDMS and the mold are stronger than the intermolecular forces within the PDMS. As a result, the PDMS climbs upwards at the edges. This is what happened in Figure 4.3. It is basically not possible to accurately remove these raised edges to be able to use the part.

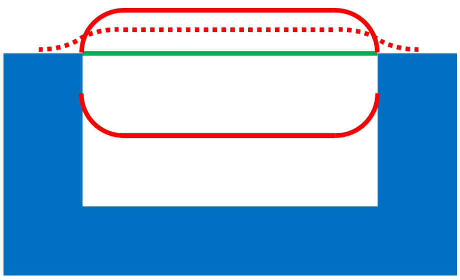


Figure 4.4: Schematic cross section of filling levels

For the solid red line on top the level of PDMS is slightly higher than the mold. Surface tension within the PDMS tries to keep the PDMS together. When the PDMS is put in the oven to cure, the surface tension breaks and the PDMS starts overflowing (dotted red line). If the overflown PDMS covers the main channel or the cylinders, it needs to be removed before it can be used. These parts need to stay open because they are connected to the membrane layer. Otherwise the unwanted PDMS blocks the membrane. Similar to removing the raised edges, removing overflown PDMS accurately is extremely hard to do.
At last we have the green line. Here the mold is filled perfectly to the same level as the mold, so no meniscus will form. This is the level to aim for. It was hard to see if this level was reached. The reflection of the light was used to help with this. A meniscus will bend the light as can be seen in the slightly underfilled mold in Figure 4.5. When the mold is filled with just the right amount of PDMS, a nice even reflection of light can be seen at the surface (Figure 4.6).

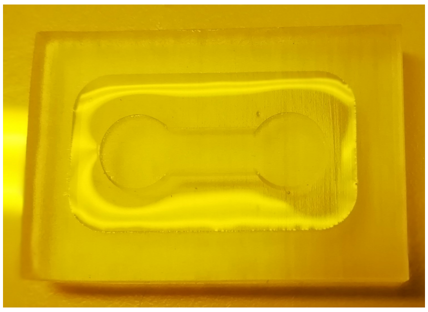


Figure 4.5: Slightly underfilled mold

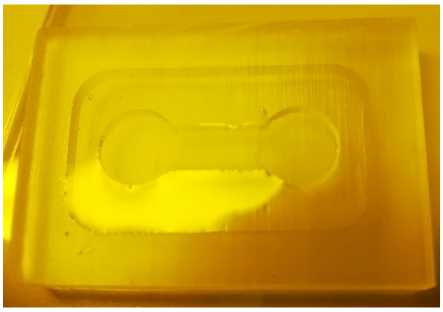


Figure 4.6: Perfectly filled mold

4.1.2 Inversing groove direction main channel

As mentioned in section 3.5, there was no documentation on why the main channel had the shape that it did. The PDMS mold should be able to make two separate layers. Therefore, it is required that the PDMS can pin the first layer in the middle (red dotted line in Figure 4.7). The original groove of the main channel does not pin the liquid, that is why the groove is inversed (Figure 4.8).

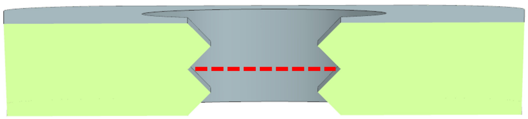


Figure 4.7: Original groove in PDMS part

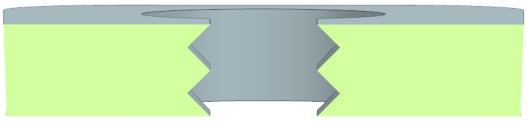


Figure 4.8: Inversed groove in PDMS part

4.1.3 Groove in cylinders

During the filling of the resin mold it could be observed that the level of the liquid in the cylinder rises to a level above the pinning point of the first layer. The liquid tries to keep itself together using its surface tension. When the forces from the increasing water column break the surface tension, the liquid floods the main channel. The liquid flow is uncontrolled and goes in both the first and second layer. A smoother transition between the main channel and the filling cylinders is needed. This transition is made by continuing the groove from the main channel into the cylinders. The surface tension can no longer pin the liquid in the cylinder and the liquid will enter the main channel before it can rise above the first layer.

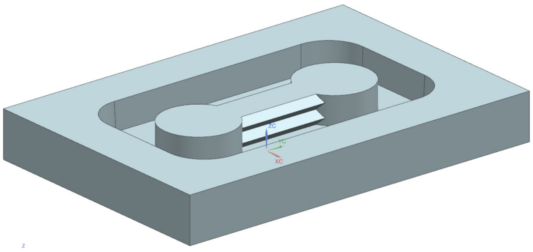


Figure 4.9: Only groove in main channel

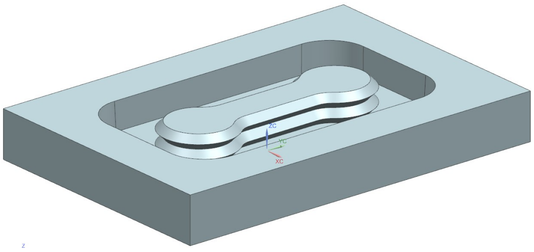


Figure 4.10: Groove continues in cylinders

4.1.4 Adding nutrient channels

Most of the molds tested so far have a total height of 3mm. To use this mold in research it should be scaled down. The maximum height of a single layer should be around 0.4mm, so a total of 0.8mm. This is because there are multiple layers of cells, which are fed from the top layer. The nutrients will then sink through the material to the layers beneath. With a layer height more than 0.4mm it becomes difficult to reach the lower cells. Making a mold with a total layer height of 0.8mm is not a problem, but making the part out of PDMS is. With the slightest filling error the channel is ruined. The limiting factor for the layer height is being able to feed the cells in lower layers. Additional channels were added on both sides of the main channel to be able to feed and provide stimuli to the cells more locally. These channels are referred to as the nutrient channels. If this would work, the layer height does not have to be reduced to 0.4mm. The cells can be stimulated from the side, which gives more vertical space to work with. The height of a single layer can become 1mm instead of the previous 0.4mm. For the first experiments with nutrient channels the total layer height stays at 3mm, but when evaluating the mold it will be kept in mind that the design needs to be scaled down in the future.

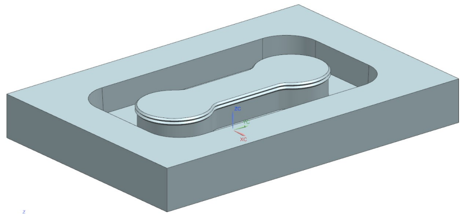


Figure 4.11: Total layer height of 0.8mm

Making nutrient channels has some restrictions. There are several ways to make a mold [10]. However, it is strongly discouraged to make a design that consists of multiple PDMS parts that need to be connected together. This is because of the difficulties of aligning parts with detailed features accurately, which is a whole separate project on its own [11].
Another restriction is that you cannot make holes or loops in you mold design, since this will create rings of PDMS that cannot be taken out without tearing the PDMS (Figure 4.12 and Figure 4.13). As a consequence, the nutrient channels should stay connected to the main channel and the cylinders everywhere.

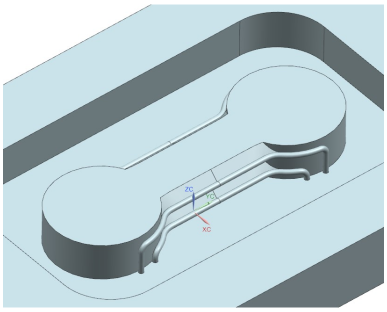


Figure 4.12: Nutrient channel makes contact with the main channel everywhere

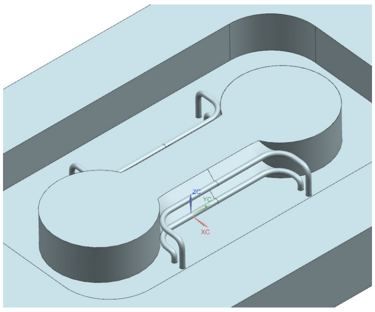


Figure 4.13: Nutrient channel is not connected to the cylinders, creating loops

The first design with nutrient channels is shown in Figure 4.14 and Figure 4.15, which was the first test to see if the printer could handle these small features. The diameter of the cylindrical channels is 0.4mm. The printer has some recommended minimum dimensions for your prints [9] and [12], these will be put to the test as will be discussed later on in this chapter. The first test was successful, so more complex models could be tested.

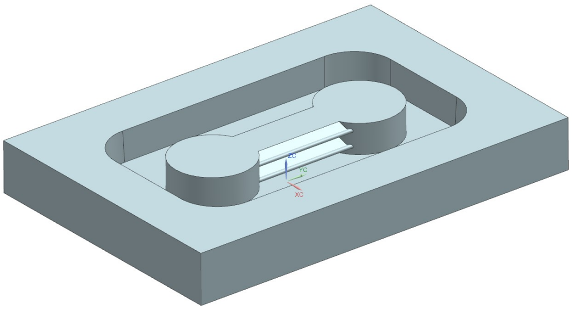


Figure 4.14: First test with nutrient channels

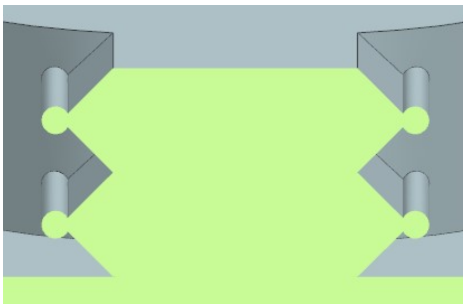


Figure 4.15: Cross section of the main channel

The next step is to add inlets for the nutrient channels, otherwise the nutrients cannot get into the channels. When looking around the lab most other experiments used setups where inlets are placed on the top side of the setup for easy access. Based on these setups, it was chosen to make the first inlets on the top side as well. The first model with inlets is shown in Figure 4.12. This model did not yet have the groove transition in the cylinders as discussed in subsection 4.1.3, so it had the same filling issue as before. Both features (groove in cylinders and nutrient channels) need to be combined in one design. For this design the inlets were placed as far away from the main channel as possible, so that the inlets do not affect the groove transition. One minor other detail is the diameter of the nutrient channel. The first test used a diameter of 0.4mm. The smallest pipette tip available to fill the channels had a diameter of 0.8mm, so in order to fit the pipette the channel diameter needs to be increased to 0.8mm.
Implementing these adjustments resulted in two designs: one with circular nutrient channels (Figure 4.16) and one with rectangular nutrient channels (Figure 4.17). The PDMS parts made with these molds can be seen in Figure 4.18 and Figure 4.19. The circular channels have a diameter of 0.8mm, the rectangular channels have a height of 0.2mm and a width of 1mm.

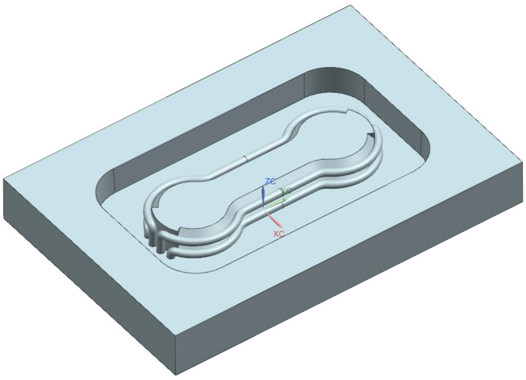


Figure 4.16: Resin mold with circular nutrient channels

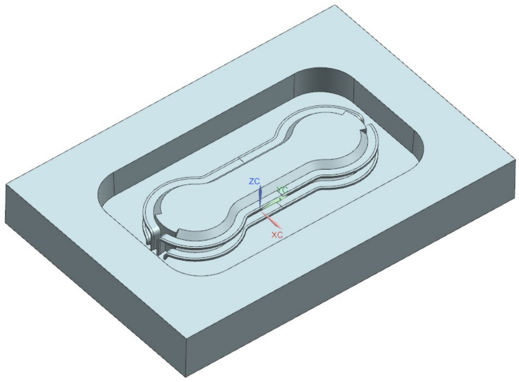


Figure 4.17: Resin mold with rectangular nutrient channels

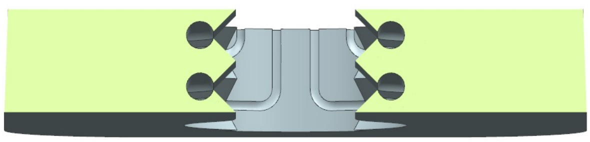


Figure 4.18: Cross section PDMS part with circular nutrient channels

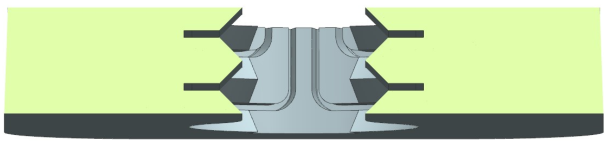


Figure 4.19: Cross section PDMS part with rectangular nutrient channels

Filling these molds was much more difficult than the other molds. Air bubbles got stuck in both molds, but especially in between the circular channels. Figure 4.20 shows where most of the air got stuck. Remember that the PDMS part is flipped when it is taken out of the mold. So although the red arrows point to the material on top of the channel, during filling this is the material underneath the channel. In these locations air bubbles try to rise to the surface but cannot go anywhere. More tests were done with this design, but all of them had similar problems. It was either with bubbles stuck in the PDMS or PDMS parts that broke off because the PDMS could not reach there resulting in parts that were too thin. The rectangular channels looked more promising, so it was decided to continue with this design for now.

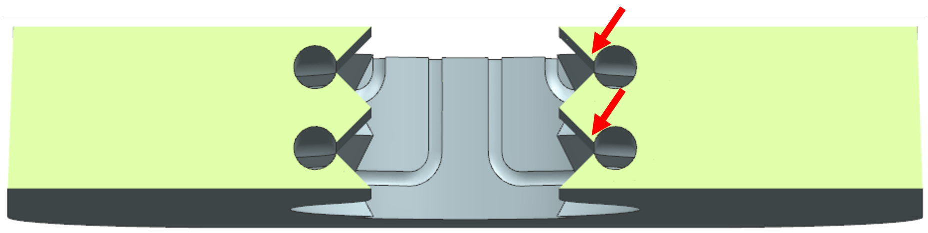


Figure 4.20: Red arrows indicating locations were the air gets stuck

One last design that was proposed was a design with a nutrient channel in the middle. The previous two designs had a nutrient channel placed in the middle of each layer, because this was convenient with the current shape of the groove. However, it would be nice to stimulate the middle layer of cells directly from the side. What made this difficult is that the middle of the groove was also the point that should pin the first layer, so the shape should stay the same. A design was made where the nutrient channel was connected to the main channel in the middle (Figure 4.21 and Figure 4.22). This design was really pushing the limits of the printer, with dimensions equal to or sometimes even just below the recommended minimum thicknesses by Formlabs [12]. Unfortunately, during the printing the thin walls separating the main channel and nutrient channel fused together, so it became just a block of resin. This design was set aside for now. The problem with the air bubbles had to be fixed before we could continue with experimenting with different designs.

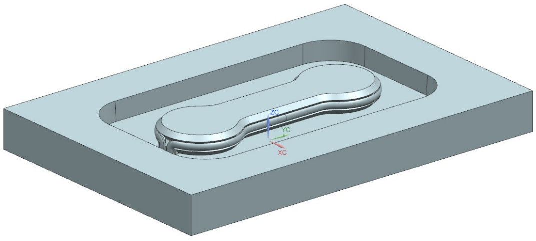


Figure 4.21: Resin mold with nutrient channel in the middle

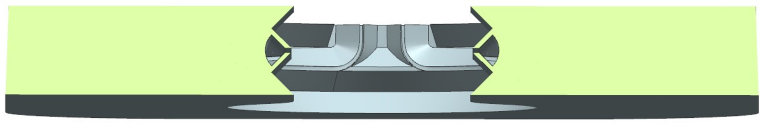


Figure 4.22: Cross section PDMS part with nutrient channel in the middle

4.1.5 Vertical filling

With the addition of the nutrient channel the design became more complex. More details meant more places where air bubbles could get stuck. You can make air bubble free PDMS by removing them when the PDMS is already filled into the resin mold or by trying methods to prevent bubbles from forming in the first place. Unfortunately, there are not a lot of ways to prevent bubbles. The standard way to fill is to pour the PDMS directly out of the container that was used in the mixing machine. Some other filling methods were tried where you can fill the mold slower and more controlled, so the PDMS has time to reach every corner. An example is the use of a syringe or a pipette. These introduces additional bubbles when they are used, so they make it worse. Another option is using a glass mixing stick where the tip is submerged in PDMS, so you can fill the mold one droplet at a time. This method was never meant to fill the complete mold, just the detail. However, it was not working better than just slowly pouring the PDMS out of the container. One more idea was to heat up the PDMS in an attempt to lower its viscosity, in order to flow easier through the mold. This was not allowed. PDMS cures with higher temperatures, which might cause local solidification. For research purposes the PDMS should be homogeneous, so local differences in structure are not permitted. In the end, the pouring methods stayed the main filling method.

About the removal of bubbles after filling, there were some options here as well. When bubbles appeared close to the surface, blowing on it with a pipette balloon worked most of the time. A popular method for removing bubbles within the PDMS is using the vacuum chamber which sucks the bubbles out. This was the standard method. Using the vacuum chamber after the filling became a necessary step to remove bubbles from the more complex designs (Figure 4.23). Being more often and longer in the vacuum chamber resulted in overflowing of the mold. Another method for removing bubbles could be by using mechanical vibrations. Both a vortex mixer and an ultrasonic bath were tried to shake the bubbles out. Both of them had little to no effect compared to the vacuum chamber.

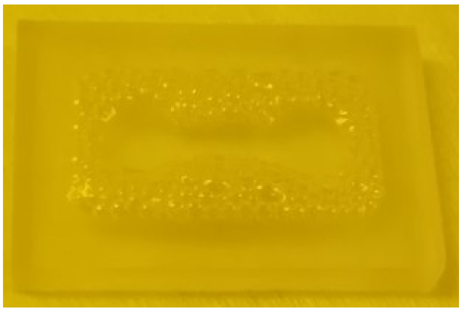


Figure 4.23: Bubbles forming on the top surface in the vacuum chamber

At this point no better solutions were found for the standard filling by pouring and standard removing bubbles with the vacuum chamber. What was left was adjusting the mold. Making a mold that can be filled vertically might solve the problem of the overflowing PDMS in the vacuum chamber. The PDMS is still going to expand, but by leaving some room on the top side for this "controlled overflow" it does not affect the quality of the channels.

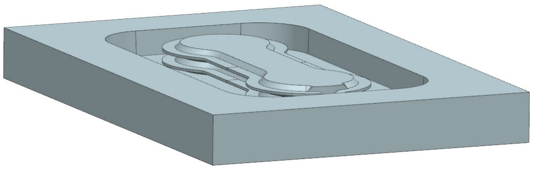


Figure 4.24: Horizontal fill mold

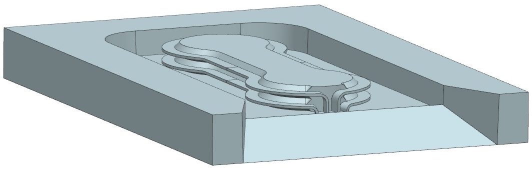


Figure 4.25: Vertical fill mold

However, switching from horizontal fill (Figure 4.24) to vertical fill (Figure 4.25) also creates new challenges. A new filling strategy is needed. Some kind of cover or lid is needed to keep the PDMS in the mold when you rotate the mold to a vertical position. The mold should be made out of two parts in order to be able to remove the PDMS part after curing. As previously discussed in Figure 4.1, the PDMS surface that was touching the printed mold is not smooth enough to make a strong bond in order to bond it to other layers. This means that the cover cannot be made out of printed resin, otherwise both PDMS surfaces cannot be bonded to other parts. A plastic called polymethyl methacrylate (PMMA) will be used to make the cover. A PMMA plate with a thickness of 3mm is cut out with a laser cutter to be placed on top of the mold.

With the use of a PMMA cover came a new challenge: finding a suitable seal between the resin and the PMMA. It turned out that making a seal to prevent the PDMS from leaking outwards is not difficult. The challenge is to prevent air from the outside leaking inwards when the mold is placed in the vacuum chamber. Switching to vertical filling became a search for the best sealing method.

Seal: double sided tape

The first try was taping the PMMA to the resin mold with double sided tape. Small strips of tape were cut and taped around the edges of the mold, as well as one strip on the main channel. One problem was that this introduced additional height to the feature on the top side of the mold, so the side that can connect to other layers. At this location it is not allowed to add height to the edges as this alters the shape of the main channel. The tape strips could not overlap, because then there would be a local height differences that negatively affects the sealing quality. They were small gaps in between the strips, this was also not ideal. Later the whole PMMA plate was covered with one piece of tape, to get rid of the gaps between the tape strips. This setup was leaking air inwards, so the next experiment was covering the whole surface with a single piece of tape to eliminate the gaps. As a consequence, the top layer of PDMS was also touching the tape, making it not smooth enough to connect it to the membrane layer later on. One idea was to place the tape in the laser cutter, to cut the outline of the resin mold and make something similar to a gasket. Eventually this plan was cancelled, since cutting the tape with the laser cutter would produce fumes that were not only harmful to inhale for humans but would also damage the laser cutter itself.

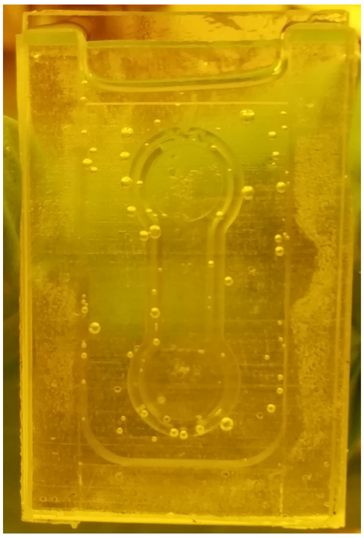


Figure 4.26: Filled mold with tape strips after curing

Figure 4.27: Filled mold with one piece of tape after curing

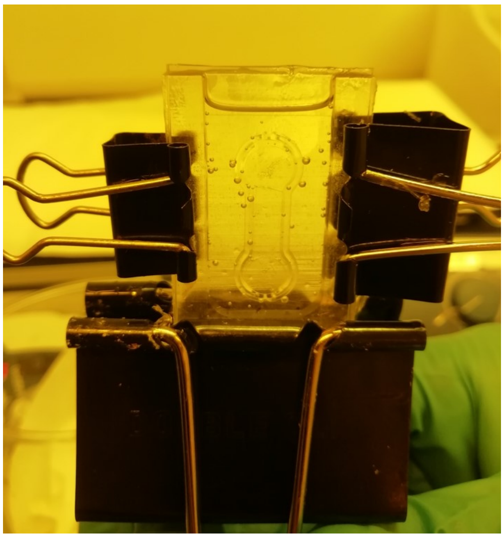


Figure 4.28: Clamps putting pressure on the tape during the curing

Seal: bolts and nuts

One of the downsides of using the clamps with the taping is that no pressure could be applied in the main channel, the most important part of the mold. When PDMS would get in between the PMMA and the main channel, it needs to be removed in a later step. As mentioned before, removing unwanted PDMS is hard to do accurately, so it is desired to prevent this from happening. A straight forward way to apply pressure to the main channel is to use bolts and nuts to connect the mold with the PMMA (Figure 4.29 and Figure 4.30). In this design, the PMMA is screwed directly to the mold with eight bolts with nothing in between. Washers are used for a more even distribution of the force applied by the bolts and nuts.

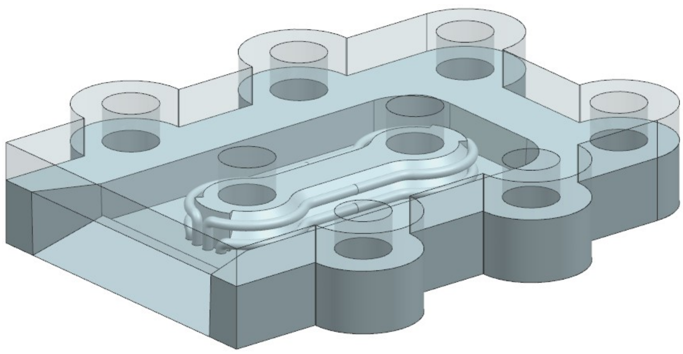


Figure 4.29: Resin mold with screw holes and PMMA cover on top

Figure 4.30: Resin mold with PMMA cover filled with PDMS

Using this number of bolts will result in an overcontrained design [13]. The problem with overconstraining is that when you have multiple parts (here bolts) that constrain the same direction or position, they might end up fighting each other when they both want to dictate/ prescribe what position the part is in. This creates unnecessary stresses in the design. This can happen for example during thermal expansion or deformation where multiple constraints contradict each other, where it is unpredictable how the forces and constraints will interact with each other.

For this design the problem is with the bolts, where they can create internal stresses when the holes in the resin mold and PMMA are not all perfectly line up. In addition, a difference in the torque with which the bolts are fastened can also create internal stresses. It can cause unwanted and unpredictable deformation, creating small slits where air can get into the mold. This is probably what happened during the filling of this mold. As can be seen in Figure 4.31, air bubbles were leaking in from the bottom and were rising to the top in the vacuum chamber. The overall quality of this PDMS part was quite good. Fortunately, the bubbles just missed the nutrient channels, so this part could be used for the first liquid testing. The part from Figure 4.31 felt like a lucky shot. For brain-on-chip research it is important that the PDMS part is completely air bubble free. This method was not consistent in making such PDMS parts, as the bubbles appeared in different places when this mold was used a second time.

Figure 4.31: PDMS part coming out of Figure 4.30

Seal: adhesive layer

One option for a seal is using an adhesive layer to temporarily bond the PMMA to the mold. After the PDMS is filled and cured the adhesive layer needs to be destroyed to remove the PMMA from the mold. For this experiment an adhesive called Norland optical adhesive 81 (NOA81) [14] is applied to the outer edge and main channel of the resin mold, then the PMMA plate is placed on top. NOA81 cures when it is exposed to UV light. After the UV curing it looked like the PMMA was secured to the mold. You could not pull the two pieces apart. Unfortunately, this sealing method was also leaking (Figure 4.32). When the PDMS was cured and the PMMA was taken off, it was found that the adhesive was still a liquid in most places (Figure 4.33). It should have been hardened out after the UV exposure. It is unclear what went wrong, since the NOA81 seemed to bond the PMMA and resin mold together before the PDMS filling started. Applying the adhesive to the main channel was difficult, so it no surprise that that part started leaking as well. This option was not preferred anyway, since the adhesive added an unknown height the the main channel, just like the tape strips did.

Figure 4.32: Air bubbles in the mold with NOA81

Figure 4.33: After curing the PDMS the NOA81 was still mostly a liquid

Seal: O-ring

The next idea was making a rubber sealing. A rubber O-ring was cut to make a U-shape. A groove was made in the outer edge of the mold to fit the rubber ring (Figure 4.34). The dimensions of the groove were chosen so that the rubber ring with slightly stick out above the mold, so it needed to be compressed by the PMMA to make a good seal. This meant that some more force had to be applied than the office clamps that were used before. It was decided that the best way to apply this force is by using bolts again. The aim was to use less bolts than in the bolts and nuts design, since the bolts still overconstrain the design. Two designs were made, one with a thick and one with a thin O-ring (Figure 4.35 and Figure 4.36). Both were leaking, as can be seen by the air bubbles around the rubber as well as around the main channel.

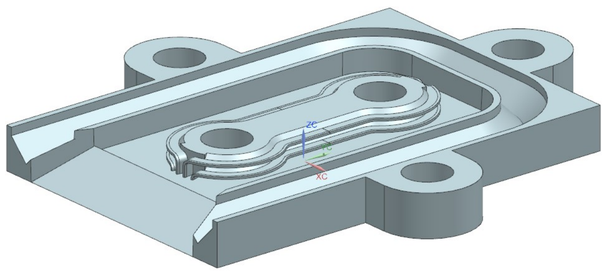


Figure 4.34: Resin mold for an O-ring

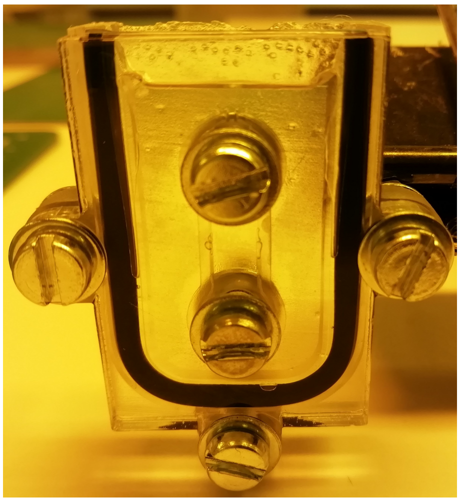


Figure 4.35: Air bubbles with the thick O-ring

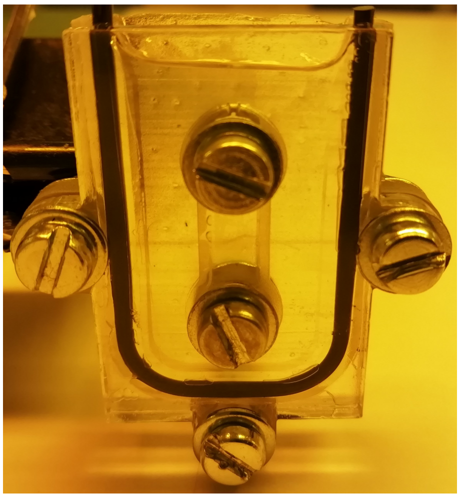


Figure 4.36: Air bubbles with the thin O-ring

Upon closer inspection of the mold, it was found that the rubber ring was only properly compressed close to the bolts. Gaps between the resin and the PMMA could be seen in the corners (red arrows in Figure 4.37). When the PMMA was taken off after curing the PDMS was leaking almost everywhere, except for the rubber close to the bolts. This can be seen in Figure 4.38, where especially around the top and bottom bolt hole the difference in light reflections shows that those are the only places where PDMS did not leak. In this experiment, it looked like the resin mold was warping more than the PMMA plate, suggesting that the resin is the weakest part here.

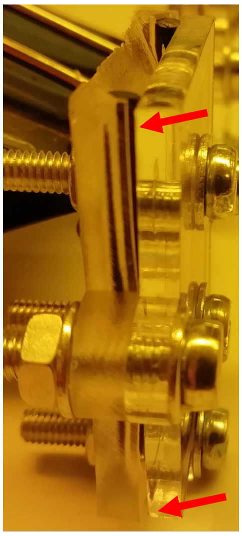


Figure 4.37: Side view of the O-ring design

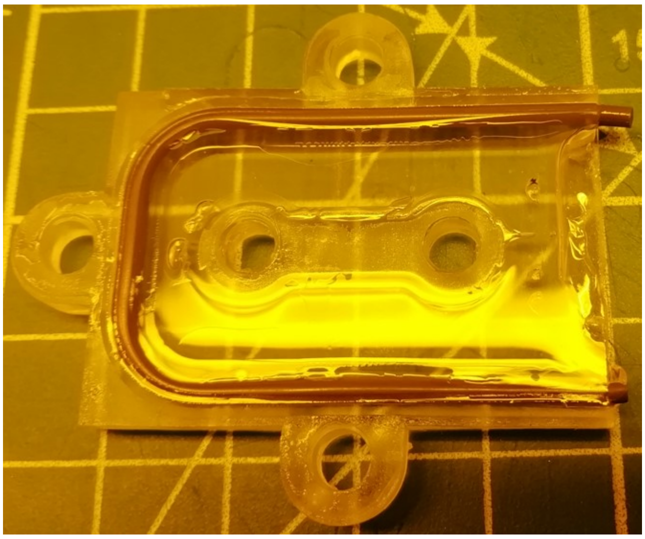


Figure 4.38: O-ring design after curing

Seal: PDMS layer

For the PDMS seal a layer of PDMS will be bonded to the PMMA plate. This resin layer will then be pressed to the resin mold creating the seal. To make this layer the PMMA is first cleaned with ethanol. The clean PMMA is placed in the spin coater, where about half of the area is covered with PDMS. The spin coater spreads out the PDMS to an even layer. The PMMA with PDMS layer is cured first, after that it gets an silanization treatment over night. The silanization makes a protective layer on top of the PDMS, so that the PDMS that will fill the mold will not connect to the PDMS from the sealing layer. Because the PDMS is flexible, it acts a bit like a cushion. At the outer edge and at the main channel it can be compressed to make a good seal. Unfortunately, this seal was also leaking from the bottom.

Removing bottom bubble

Even though the air bubbles removal techniques became better, one of the bubbles that was hard to remove was the bubble on the bottom of the mold (red arrow in Figure 4.20). These bubbles are getting stuck in between the inlets of the nutrient channels. At first it was tried to solve this making wider inlets (Figure 4.40). Later on in the project the decision was made that inlets on both side are not necessary for the research experiments, so a design was made with inlets on only one side (Figure 4.41). This design has no inlets on the bottom side, so even less features that can trap air bubbles.

Figure 4.39: Piece of PDMS turned out well, except for the air bubble (red arrow)

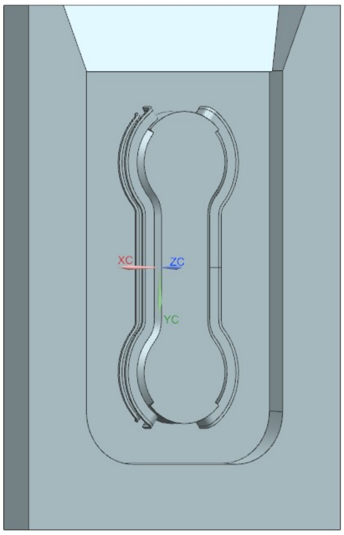


Figure 4.40: Mold with wide inlets

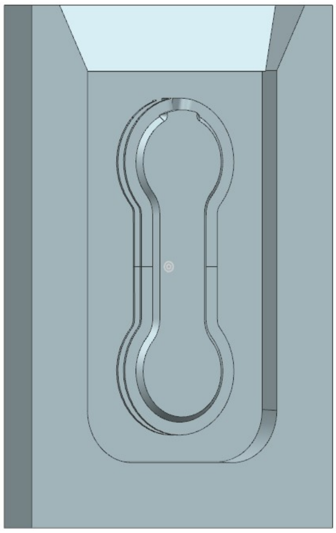


Figure 4.41: Mold with inlets only on the top

Upside down filling

One last attempt was done making a mold with a cover. It was more an upside down mold than a vertical mold. An observation made during the vertical filling was that air bubbles that get into the mold will naturally rise upwards to the surface (Figure 4.44). When they enter the mold from the bottom, it is likely that these bubbles will get stuck in the features of the main channel. This might be solved with the upside down design. As can be seen in the previous attempts it is hard to prevent leaking. The problem is also solved when the bubbles that do come in do not in or close to the features. Figure 4.42 shows in bright blue the PMMA plate, Figure 4.43 shows the total assembly after filling the PDMS and curing. The design in upside down because normally the smooth side of PDMS will be at the top, but here it will be on the bottom touching the PMMA. The idea is that bubbles that enter the mold will still rise up, but since they come from the side they will rise up close to the side not interfering with the channels (Figure 4.45. When it is a problem that there are any bubbles in the PDMS, you can always make the outer edge a bit wider and cut of the edges of the PDMS that has bubbles in it. The design is filled from the top through the four openings in the resin mold. On the top surface a meniscus will form as discussed in subsection 4.1.1, but here that is not a problem anymore since the top surface is not the surface that will bond to other layers.

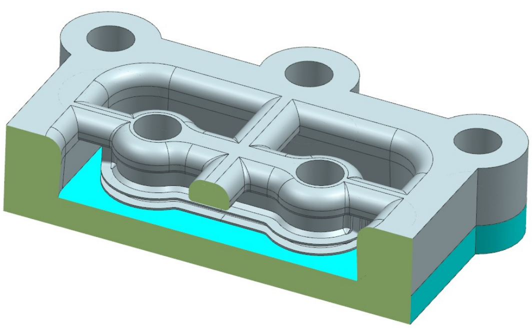


Figure 4.42: Cross section mold for upside down filling

Figure 4.43: Mold for upside down filling assembled

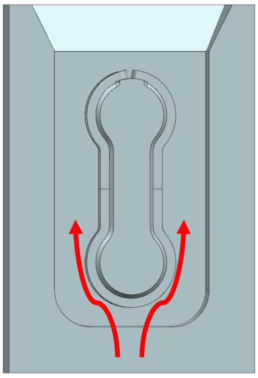


Figure 4.44: Air getting in the mold from the bottom rises around the feature

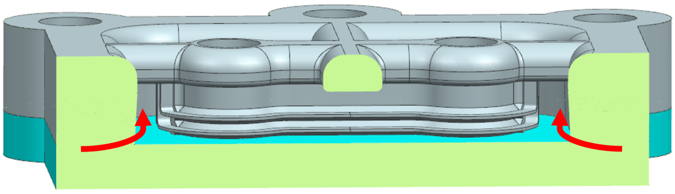


Figure 4.45: Air getting in the mold from the side not interfering with the feature

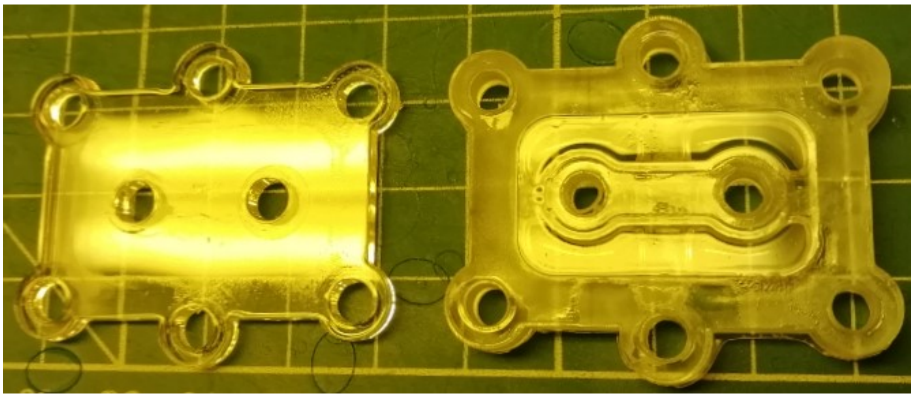


Figure 4.46: Upside down mold taken apart after curing, smooth PDMS side facing upwards

4.1.6 Back to horizontal filling

Making mold that could be filled vertically did not give the desired improvement in quality, especially not compared to the increase in difficulty for filling the mold. It was decided to switch back to horizontal filling. For the last models in this project, a number of smaller adjustments was implemented at once in the final designs, based on the lessons learned from the previous designs. The most important change is the switch from using 7:1 (base : curing agent) mass ratio PDMS to 10:1 PDMS. This was done to make PDMS with a lower viscosity, which can fill the mold easier around the small features. It made the filling easier, which meant that it needed less time in the vacuum chamber so no overflowing PDMS. Next are some changes to make it easier to get the PDMS out of the mold. The main channel is lifted a bit (Figure 4.47). This creates a PDMS part that is a bit thicker on the bottom, which decreases the risk of tearing the part when peeling it out of the mold. On received recommendation was the use of a mold release agent called Mann^{TM} Ease Release^{TM} 200. This is sprayed on the resin mold before each fill. As the name suggests it helps releasing the PDMS by making it stick less to the resin mold. The last feature that is changed is the inlets. The nutrient channels are rectangular shaped, so are the inlets. The last modification is a transition from from rectangular nutrient channels to circular inlets (Figure 4.48). This way the design has the benefits of the rectangular channels and at the same time it has inlets that can hold tubes to connect it to the liquid pump.

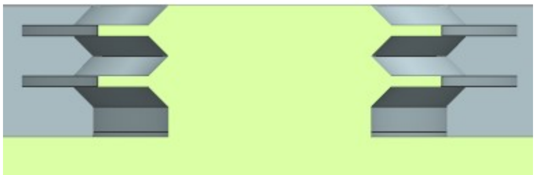


Figure 4.47: Cross section mold raised main channel

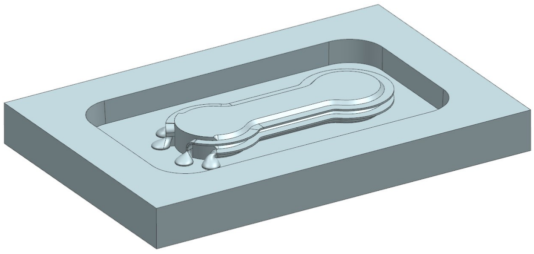


Figure 4.48: Mold with rectangular nutrient channels and circular inlets

4.1.7 Print orientation

Formlabs has their own software to prepare prints called Preform. It turns a CAD model into instructions that the printer can understand. The position and orientation of the model in Preform (and thus the printer) affects the quality of the print. Preform want to add a support structure around parts of the print where it thinks the print might break off during printing. When printing the models, it is desired that this support structure is not connected to vulnerable parts like the nutrient channels, as there is the risk they get damaged or break off when removing the support. For example, the rectangular nutrient channels are what is referred to as 'overhang', which means that there is a horizontal piece that is only connected to the rest of the model on one side. A piece like that has a risk of failure (by falling down) when in sticks out too far. According the the Formlabs' design guide [12], the maximum unsupported overhang the printer can handle is 5mm. With the nutrient channels' width of 1mm this should by fine. However, the models printed in this project are not printed under standard conditions. The 5mm overhang is not based on a feature with a thickness of 0.2mm where the connection point to the rest of the part is under an angle, making that point even weaker. What the printer could handle was a combination of an educated guess and trail-and-error. For example, the overhang is measured relative to the horizontal XY-plane of the printer. By rotating the design, it is sometimes possible to reduce the horizontal overhang to a value that can be printed without support.

Figure 4.49 and Figure 4.50 show the same model prepared in Preform in different orientations. (Although this paragraph is about using resin Clear V4, Figure 4.51 to Figure 4.54 show designs make with the resin Tough 1500. Since both resins behave similarly, the described phenomena happens to models made out of Tough 1500 as well as Clear V4 resin.) Figure 4.49 is placed flat on the surface, while Figure 4.50 is placed under an angle and therefore needs a support structure. Apart from the orientation, the rest of the settings are the same. The additional material for the support as well as the additional vertical layers needed drastically increase the printing time (just over 1 hour versus close to 8 hours when using Clear V4 resin). Notice that the chosen orientation in Figure 4.50 makes use of the features of the model. The inlets of the nutrient channels are directed downwards, so that the nutrient channels can support on the inlet which makes additional support unnecessary. This only works in this orientation. When this model was placed on its long side instead of its short side the inlets could not provide support and a support structure is needed. For the horizontally printed part Preform suggested support, lifting the part of the build platform. This did not improve the quality of the print, because sometimes the parts were not completely flat on the top. This was a issue with the vertical filling molds, where a flat plate of PMMA was touching a slightly bend mold. Fortunately, the PMMA was flexible enough to compensate for the bend and bend along in most cases.

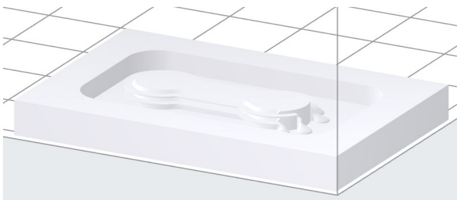


Figure 4.49: Printing time: 1 hour 5 minutes

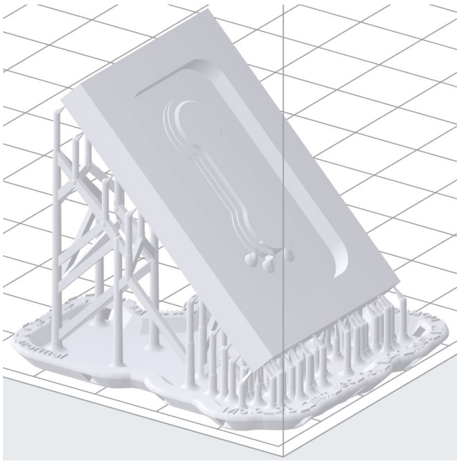


Figure 4.50: Printing time: 7 hours 50 minutes

When the models came out of the printer, the horizontally printed part looked fine (Figure 4.51). The part printed under an angle (Figure 4.52) did not. It looked like a failed print since the nutrient channels of this part were warped and looked like a sheet of corrugated metal. Surprisingly, this curing process got rid of the warping (Figure 4.54). The horizontally printed part also did some warping, but here it looks like the warping negatively affected the quality of the part (Figure 4.53). The nutrient channels look thicker from the side, suggesting that these might be bent. It is hard to see on the pictures, but if you look closely it looks like the nutrient channels are bend upwards, as shown in the schematic of Figure 4.55. One can argue how big of a problem this is. On an absolute scale the bend is small, but on a relative scale for the size of the design the bend might be (too) big. For this specific design printing horizontally greatly reduces the printing time. Because of the horizontal printing, the horizontal top surfaces are a little smoother than the one printed under and angle. However, both are still not smooth enough to make a PDMS part that can be bonded at the surface that touched the mold. About the warped nutrient channels, (and therefore less space in between them), this might cause problems with the filling and removing of PDMS. It might be harder for the filling because it is harder for the PDMS to get there, harder for the removing because the PDMS is thinner there which makes it easier to tear. For each part it should be evaluated if printing orientation result in a part with sufficient quality.

Figure 4.51: Printed horizontally, before curing

Figure 4.52: Printed under angle, before curing

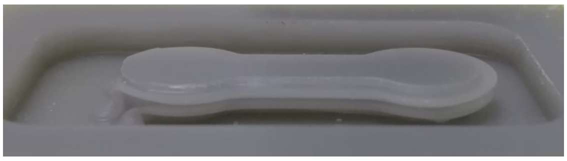


Figure 4.53: Printed horizontally, after curing

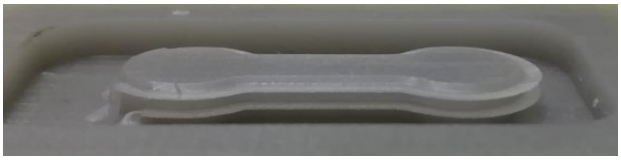


Figure 4.54: Printed under angle, after curing

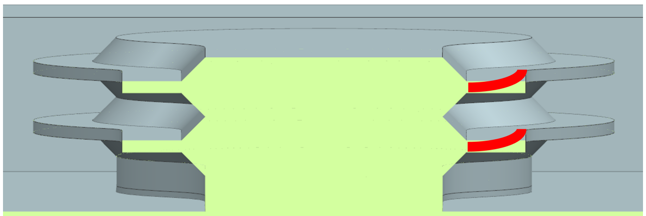


Figure 4.55: Cross section with in red the warped nutrient channel

4.2 Filling the PDMS mold

When the first PDMS models were made, the filling experiments could start. One of the first experiments was to check if the levelled (subsection 4.1.1) mold could make a PDMS part that was flat enough so that it could be bonded to a glass microscopic plate (Figure 4.56). When it was bonded the channel was filled to see if the bonding would not leak. It did not, which showed that levelling the mold worked to make a flat PDMS surface.
After that bonding was leak free the mold was filled again but this time to see how the mold fills up with the liquid. With this experiment was discovered that the original groove did not pin the liquid (Figure 4.8) and that because of the sharp edge of the cylinder the liquid builds up in the cylinder before it enters the main channel (subsection 4.1.3).

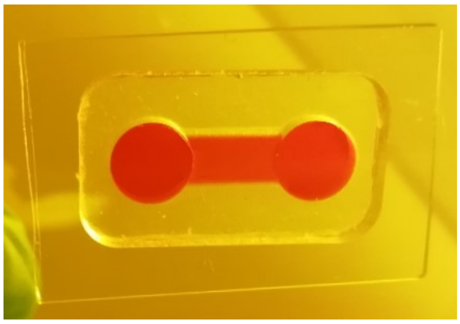


Figure 4.56: Bottom view: test with red liquid to see if the bonding is not leaking

The experiments that came after this are mainly focused on filling the nutrient channel. In the experiment colored gelatin is used as a substitute for the much more expensive hydrogel. Gelatin and hydrogel have similar mechanical properties that are important in the experiments, for example a similar viscosity. The initial idea was that the liquid filling the main channel had a viscosity high enough that it would prefer staying in the main channel instead of going into the small nutrient channels. The opposite was the case when the mold was filled with the gelatin. When the main channel was filled with blue colored gelatin from the cylinder on the right side (shown in Figure 4.57), the liquid filled up the nutrient channels (to the left side) as soon as the level of the liquid reached these channels. The capillary force pulling the liquid into the channels is stronger than the surface tension that want to keep the liquid together. As a result, the nutrient channels are filled before the liquid in the main channel can catch up. This method would not work for filling the two channels separately. Moreover, Figure 4.57 nicely shows how the blue liquid is pinned halfway of the total height, so where the first layer should be pinned.

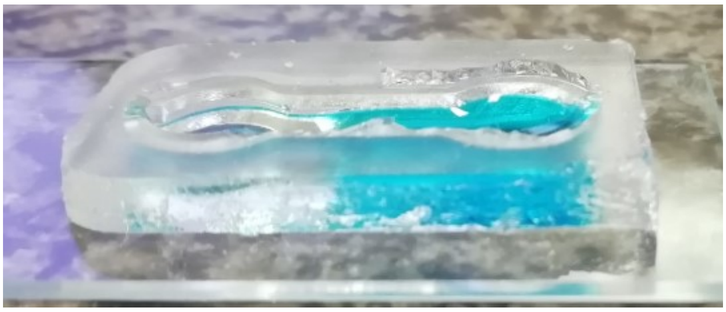


Figure 4.57: PDMS filled with blue colored gelatin from the right cylinder

The gelatin going into the nutrient channels does not have to be a problem. Instead of filling the main channel first, the nutrient channels can be filled. The way to do this is by filling up the whole channel, so main channel and nutrient channel. Here colored water is used instead of the colored gelatin, because in the real experiment there will also be no hydrogel in the nutrient channels. After that the liquid can be removed from the main channel. The liquid in the nutrient channels will stay behind, due to the capillary force pulling it in the channel. The result of this filling with a red colored liquid can be seen in Figure 4.58 and Figure 4.59.

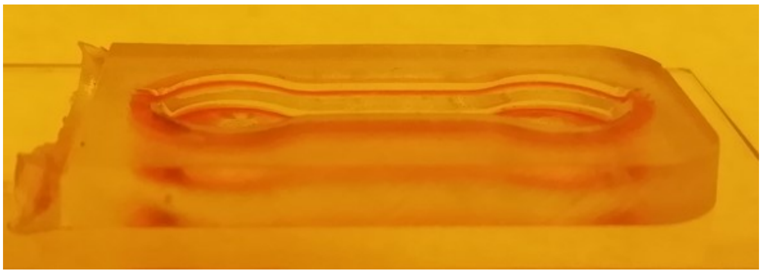


Figure 4.58: Side view red liquid in nutrient channels

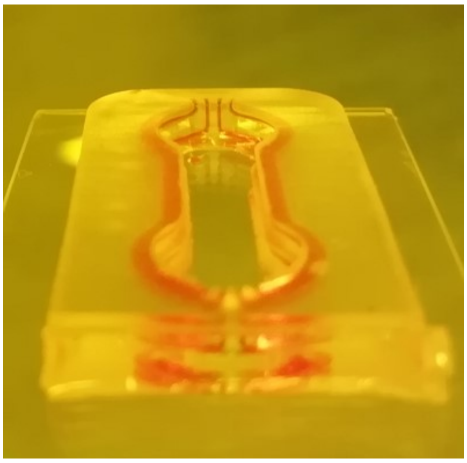


Figure 4.59: Red liquid in nutrient channels

The plan was that now the nutrient channels are already full, the gelatin filling the main channel cannot get into the nutrient channel. After the hydrogel (or gelatin) is set, the liquid can be removed and you should end up with open nutrient channels. Experiments showed it did not work that way. First the nutrient channels are filled with red colored water (Figure 4.60). From the moment the blue colored gelatin fills up to the level of the nutrient channels, it starts pushing out the red liquid (Figure 4.61).

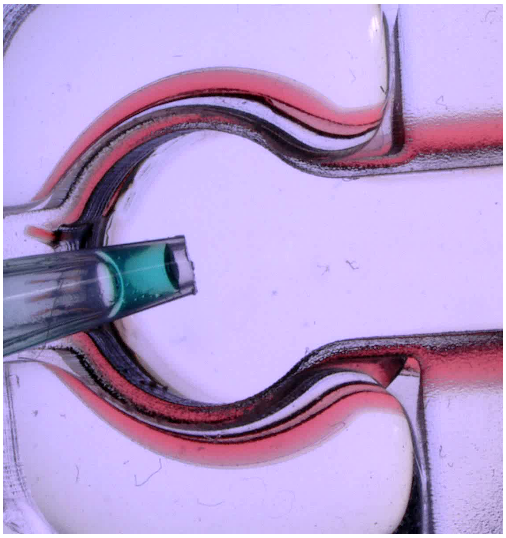


Figure 4.60: Top view with red liquid in nutrient channels before filling

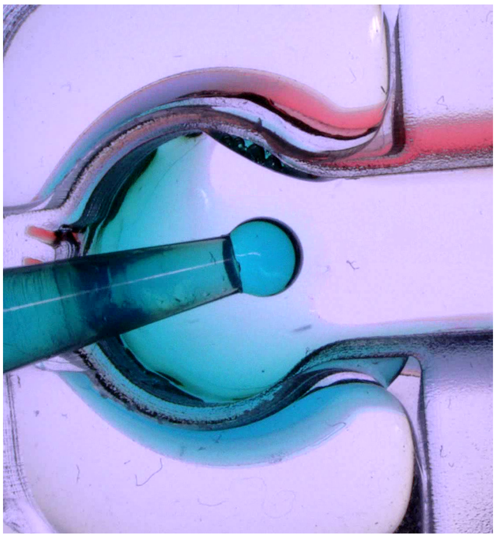


Figure 4.61: Top view filling main channel with blue gelatin

Since pushing out the red liquid happens almost instantly, there is not much to do about it. Changing the filling speed or filling position makes no difference. In the next experiment the idea was to create a continuous flow of liquid in the nutrient channel, which would help preventing the mixing of liquids when the main channel will be filled. For the first attempt to make this the Chemyx Nexus 3000 syringe pump (Figure 4.62) was used. A syringe pump can compress or expand a syringe automatically, making it a more controlled filling method. The metal block in the middle can move both to the left and right. In the figure both syringes are on the same side, but if you bring one to the other side one syringe will be compressed while the other will be expanded. In theory one syringe could push the liquid, while the other pulls on it. The pushing part went well. The pump was set to a flow rate of $50\mu L$ per minute. Figure 4.63 and Figure 4.64 show the bottom nutrient channel being filled with blue colored water. The time in between the figures is eight seconds. When the liquid reached the outlet it did not get sucked in the outlet tube connected to the pulling syringe. Initially there was nu liquid in any of the channels, so the pulling syringe was sucking air until the liquid reached the outlet. Even then removing the liquid was not efficient or sometimes not working at all. What is not helping is that as long as there is nothing filling the main channel, the nutrient channels are open channels. Trying to suck the liquid out using an underpressure will be more difficult with open channels. The result was that the nutrient channel started to overflow when the liquid reached the outlet. The syringe pump was not specifically made for pumping around liquid, so another pump was tried.

Figure 4.62: Syringe pump

Figure 4.63: Pumping blue colored water using a syringe pump

Figure 4.64: Pumping blue colored water using a syringe pump 8 seconds later

The other pump was a peristaltic pump, the ibidi pump system. This pump consisted of two syringes that where wired so that they could pump easily in both directions (Figure 4.65). If you want to pump liquid from the right syringe through an actuator (here the inlet and outlet of the nutrient channel) to the left syringe the liquid should start with the black arrow and follow the path of the blue arrows. However, even though the pump was installed as suggested in the manual [15], the liquid was never pumped around through the actuator because it used the shortcut path of the black and red arrow. Switching the tubes of the setup did not get rid of the shortcut.

This was the final experiment that was done in this project. The adjustments to the peristaltic pump could not get it to an operating condition, so this experiment could not provide results fot this report.

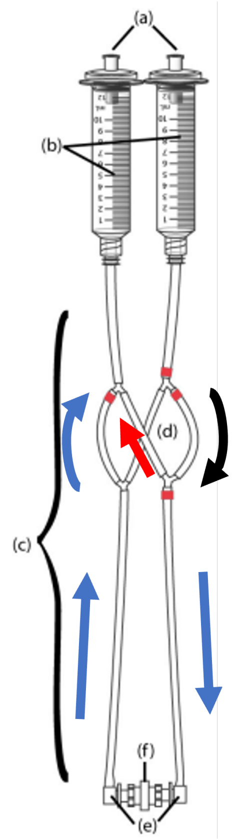


Figure 4.65: Schematic overview pump direction

5 Conclusion

With the latest manufacturing and filling method for the resin mold, PDMS pieces can be made that meet the desired quality requirements for research. What might be even more important, the quality is more consistent now.
The first modifications to the design, inversing the groove shape and making the groove in the cylinders, were both necessary steps to pin the liquid in the correct way. When it comes to removing the bubbles from the PDMS, changing the mass ratio of the PDMS was way more effective than finding ways to seal the mold with a cover.
The challenge that is left is how the main channel and nutrient channel can be filled effectively to complete the setup. In chapter 6 some ideas are suggested on how this filling issue can be solved.

6 Future outlook

The focus during the liquid filling experiments was on methods that would work for cell culture. Some ideas might have been turned down a bit too fast. To find a solution for filling the channels it might be useful to forget about the cell culture for a moment, to come up with ways to fill. A personal hypothesis is that it might work when using two liquids with different properties, for example different densities. When the nutrient channel is filled with a relatively heavy liquid it might not be pushed out as easily. Other differences could be water and a hydrophobic liquid. In one experiment the main channel was filled with olive oil (Figure 6.2), but the result let to some discussion. It is unclear if the oil fills the nutrient channel, or if this is just the reflection of the light. Either way, it is interesting to see what would happen when the channels are filled olive oil and water. Another suggestion is looking for liquids where the ratio between cohesive forces (attraction to molecules of the same material) is relatively high compared to the adhesive forces (attraction to molecules of a different material) [4]. If the liquid does not want to interact with other materials it is less likely that it will fill up the nutrient channels. For materials that have to be used (for example the hydrogel) it can be looked into how the adhesive forces with PDMS can be reduced to achieve a similar effect.
What could also work is using some kind of salt or sugar that can crystallise in the nutrient channel. After the nutrient channel is filled the salt can be rinsed away. For this suggestion (or in general) it might be nice to make a mold of the main channel without nutrient channels. This mold can be inserted into a piece of PDMS to fill the nutrient channels without them overflowing (Figure 6.1). When the filling issues are solved, it might be worth it to revisit older designs. For example the middle channel design might be useful for supplying nutrient directly to the middle layer.

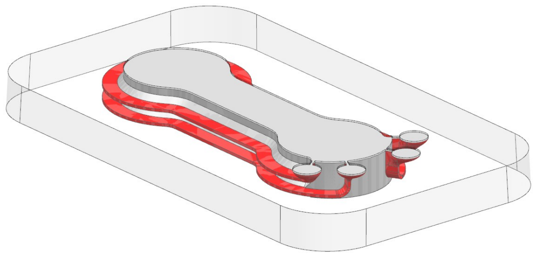


Figure 6.1: Mold (grey) of the main channel to fill the nutrient channels

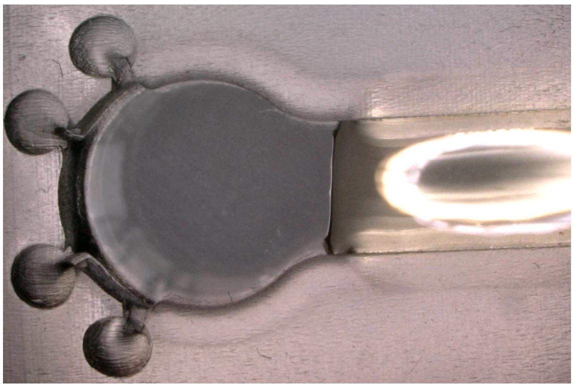


Figure 6.2: Filling the main channel with olive oil

Finally, a last mold was made. All the previous mold had vertical inlets that were accessed from the top. For a new experiment a design was made with horizontal inlets, to test whether or not this would improve the inlets for the nutrient channel (Figure 6.3). This design has quite some overhang, but cannot be supported because that would create loops as previously shown in Figure 4.13. It did need some support, otherwise the horizontal parts will just fall down. The minimal amount of support was added in Formlabs. For filling the resin mold the latest method was used as described in subsection 4.1.6. It was not expected that this fragile design would turn out as well as it did. Not only could the mold be printed and the support be removed without breaking it, it could be filled with PDMS and be removed out of the mold without breaking the PDMS as well as the mold (Figure 6.4). The last step of saving the PMDS part as well as the resin mold was a pleasant surprise. This design has not been tested yet but is definitely worth testing.

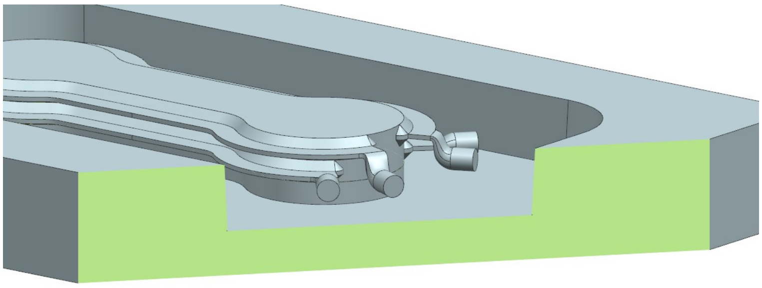


Figure 6.3: Mold with horizontal inlets

Figure 6.4: PDMS part with horizontal inlets

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A Making PDMS parts

All parts of the actuator are made from polydimethylsiloxane (PDMS) using the SYLGARD^{TM} 184 Silicone Elastomer, which consists of two components: a base and a curing agent. The mass ratio of these components affects the material properties of the PDMS and depends on the desired properties of each layer.
Making PDMS starts with measuring the correct ratio of the containers in a plastic container on a scale. These ratios are not too precise; if the ratio is off by up to 10% it is still fine. It is important that the components are mixed well and that possible bubbles will be removed from the PDMS. After combining the components into the container the mixture will be stirred by hand using a glass stick. After that, the container is put in a Thinky (centrifugal) mixer which will mix and degas the mixture. The PDMS is now ready to use.

A.1 Air channel layer

The air channel layer is made with a PDMS mass ratio of 7:1 (base : curing agent). Compared to the 10:1 ratio of other parts the 7:1 ratio makes this layer relatively stiffer. A special wafer was made as a mold to make the channels (see Figure A.1). The wafer has upstanding features, which will turn into grooves in the PDMS part. Interestingly, as you can see with the channels in the bottom of the figure they are missing some lines due to a manufacturing error, so this specific one cannot be used.
Making the air layer starts with cleaning the wafer with an air gun, to remove any dust. The wafer is then treated with oxygen plasma, with the channels facing upwards. After that the wafer gets a silanization treatment using chlorotrimethylsilane (CTMS) in a vacuum chamber overnight. The oxygen plasma makes the silanization more effective, because it temporarily opens molecule bonds in the wafer with makes it easier to bond it to the CTMS. The silanization treatment will protect the wafer during the pouring and removing of PDMS. Silanization will also be used to prepare the thin glass discs for the membrane layer as well, so these steps can be combined. A total of 15 μL CTMS will be used for the wafer and 8 discs for the membrane layer. The next day a piece of aluminium foil is folded in the shape of a low edge container just large enough for the wafer to fit in. The air channels are facing upwards. The aluminium foil will create an edge so that PDMS can be poured in. Approximately 23 grams of 7:1 PDMS are poured onto the wafer. This will cure at 65°C for at least 1-2 hours or preferably overnight. To remove the cured PDMS, an outline will be cut around the edge of the wafer. The PDMS can now be peeled off carefully. It is placed on a cutting mat with the air channels facing upwards. The channels will be covered with tape, to prevent getting dust in them before they are used. The PDMS can be cut in strips, parallel to the tape. Turn the PDMS upside down (air channels and tape facing the cutting mat) and cut the strips into individual blocks. Turning it upside down will make it easier to cut smoothly through the PDMS and at the same time makes it easier to cut the tape. The air channels are now ready for the bonding.

Figure A.1: Close up of the air channel wafer

A.2 Membrane layer

The membrane layer is made with a PDMS mass ratio of 10:1. This makes the membrane more flexible, which is desired for a membrane to be able to vibrate. The membrane layer is made using thin glass discs. The process starts by cleaning the discs with a paper towel and ethanol (and the air gun if needed). As discussed with the wafer for the air channel layer, the discs also need to be prepared with an oxygen plasma and silanization treatment. After the treatment the discs are put on a spin coater one at a time. About half of the area of the disc is covered with 10:1 PDMS. During the spin coater process the centrifugal force will spread out the PDMS evenly over the disc. This results in a membrane layer with a thickness of $10 \mu m$. The excess PDMS will fly off the disc during the spinning. The membrane will also be cured at 65°C for at least 1-2 hours or preferably overnight. The membrane is now ready for the bonding.

A.3 Reservoir layer

The design for the reservoir layer mold changes over the course of this project, but the general procedure for filling the mold stays the same. The reservoir layer is made with a PDMS mass ratio of 7:1. Near the end of the project this will change to a ratio of 10:1. If the resin mold is filled for the first time it is enough to clean the mold by blowing off any dust using an air gun. When filling it a second time or more, there might be broken off PDMS parts left in the mold. Using IPA can help to dissolve the PDMS. The air gun does not work so well here, since the PDMS is quite sticky. A disposable needle from a pipette was used to peel off the PDMS that was stuck between the details of the mold. Using tape to catch small pieces of PDMS also worked well. Now that the mold is clean it can be filled with new PDMS. Chapter 4 discusses all the issues that occurred during the filling of the mold, so they will not be discussed here again. Important is that we end up with a full mold without air bubbles in the PDMS before the curing starts. Initially, the curing would take at least 1-2 hours at 65°C. However, as the designs became more complex and more detailed it turned out this 1-2 hours was not enough anymore to completely cure the PDMS. It became necessary to cure the PDMS overnight. The next day, the reservoir layer is ready to be bonded.

A.4 Assembling layers

When you want to bond two pieces of PDMS together or want to bond PDMS to a glass microscopic slide the first step is to give both pieces an oxygen plasma treatment, where the surfaces that you want to bond are facing upwards during this procedure. When you connect the pieces you do not want any air to be stuck in between the pieces, since this weakens the bond. Normally when you put tape on an object you start at one end under an angle and slowly increase the contact area between the tape and the object where you can focus on pushing the air out from underneath. This method does not work here. You need to lay the PDMS carefully horizontally on top of the microscopic slide (the whole contact area at once), then let it rest for a second. After that gently push the PDMS down to the glass, making your way from one side to the other.
An important factor for the bonding is the surface you try to bond. As mentioned in subsection 4.1.1, the surface of the reservoir layer that was touching the resin mold is not smooth enough to make a strong bond. During a test where I accidentally trapped air between a microscopic slide and the smooth surface of the PDMS, it turned out that that was still a stronger bond than bonding the rougher surface with no air trapped. It is a shame that bonding the rougher surface does not work, since this would solve a lot of problems with filling the mold. We would not have to be as careful during the filling the mold and would not have to worry about the meniscus anymore.

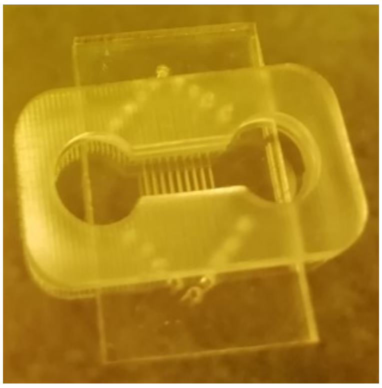

Figure A.2: Rotating the reservoir layer without adding more PDMS will not work

The assembly process of the actuator starts with bonding the air channel layer with the membrane layer. The first step is treating both layers with oxygen plasma. Place the membrane layer with the glass disc side on the table and lay the air channel layer on top with the channels facing downwards. Compared to the original actuator from Deniz (as shown in Figure 3.2a), the new actuator needs some adjustments because of the shape of the new reservoir layer. The reservoir layer from Deniz (layer C) was cylindrical and fitted in between the inlet and outlet of the air channels. The new reservoir layer does not, so it needs to be rotated 90° in order to position the main channel on top of the air channels but at the same not placing the cylinders from the reservoir layer on top of the inlet and outlets of the air channel layer (Figure A.2). As can be seen, both ends of the reservoir layer are not touching the membrane layer, resulting in no material underneath the cylinders in the reservoir layer. This setup cannot be used, since any liquid poured in will flow out. The air channel layer is a rectangular PDMS block that is limited in size by the fixed position on the wafer. These blocks cannot be made bigger without ordering a new custom wafer. Another solution is bonding more PDMS around the air channel layer. This can be done by making the same container structure as for the wafer. The combined membrane and air channel are put in the aluminium foil with the air channel layer on top (Figure A.3). PDMS with a mass ratio of 7:1 is added on top, which is the same ratio as the air channel layer. The new PDMS needs to cure 1 hour in the over at 65°C. It will now like Figure A.4.

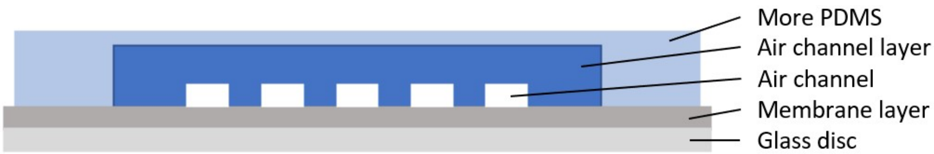

Figure A.3: Cross section of adding more PDMS to the air channel layer

Now you can carefully peel off the two assembled layers from the glass disc. Be careful, because the glass discs break easily. Place the membrane layer on top and use a 1.2mm diameter hole puncher to make the inlets for the air channels. The next step is to bond these two layers with the reservoir layer. Again, both parts get an oxygen plasma treatment and are placed on top of each other. After that the 3 layers are cured in the oven for 1 hour at 65°C. The last step is to make the inlets for the air channels go through all 3 layers. This is done by placing the air channel layer on top, placing the hole puncher in the holes that are already present in the air channel layer and extend these into the reservoir layer. Now that the inlets go through all 3 layers, the actuator is finished and ready to use for experiments.

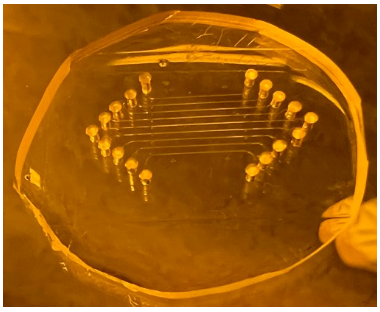

Figure A.4: Air channel layer with more PDMS around it

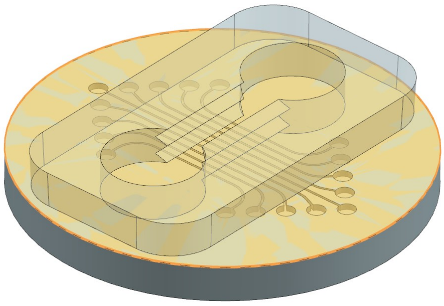

Figure A.5: The 3 layers of the new actuator