Low-Cost Microfluidic Fabrication Workshop

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Contents

1 Web-Resources 2
2 Process Overview 3
3 Workshop Overview 4
4 Microchannel Mould Fabrication 5
4.1 Items Needed 5
4.2 Process 6
4.3 Outcome 8
4.4 Heating time effects 8
5 PDMS Pouring 9
5.1 Items Needed 9
5.2 Process 10
5.3 Outcome 11
6 Scaffold Removal and Finishing Steps 12
6.1 Items Needed 12
6.2 Process 13
6.3 Outcome 15
7 Microfluidic Device Testing 16
7.1 Items Needed 16
7.2 Process 17
7.3 Outcome 17

1 Web-Resources

The workshop processes are defined in:

[1] H. Felton, R. Hughes, and A. Diaz-Gaxiola, 'Negligible-cost microfluidic device fabrication using 3D-printed interconnecting channel scaffolds', PLOS ONE, 2021, doi: 10.1371/journal.pone.0245206.

[2] H. Felton and R. Hughes, 'Open Source Microfluidic Scaffolds', 2021, Accessed: Oct. 17, 2024. [Online]. Available: https://www.protocols.io/view/open-source-microfluidic-scaffolds-biw7kfhn

[3] Low-Cost Mirofluidic Mould Fabrication - Tutorial 1, Robert Hughes Youtube.com, https://www.youtube.com/watch?v=LOcmRfbVTY4&t=24s

2 Process Overview

Figure 1 shows the 3D-printed microchannel negative fabrication process for soft lithographic device fabrication. This workshop will demonstrate steps 3-8 in Figure 1 and the assembly of a microfluidic device.

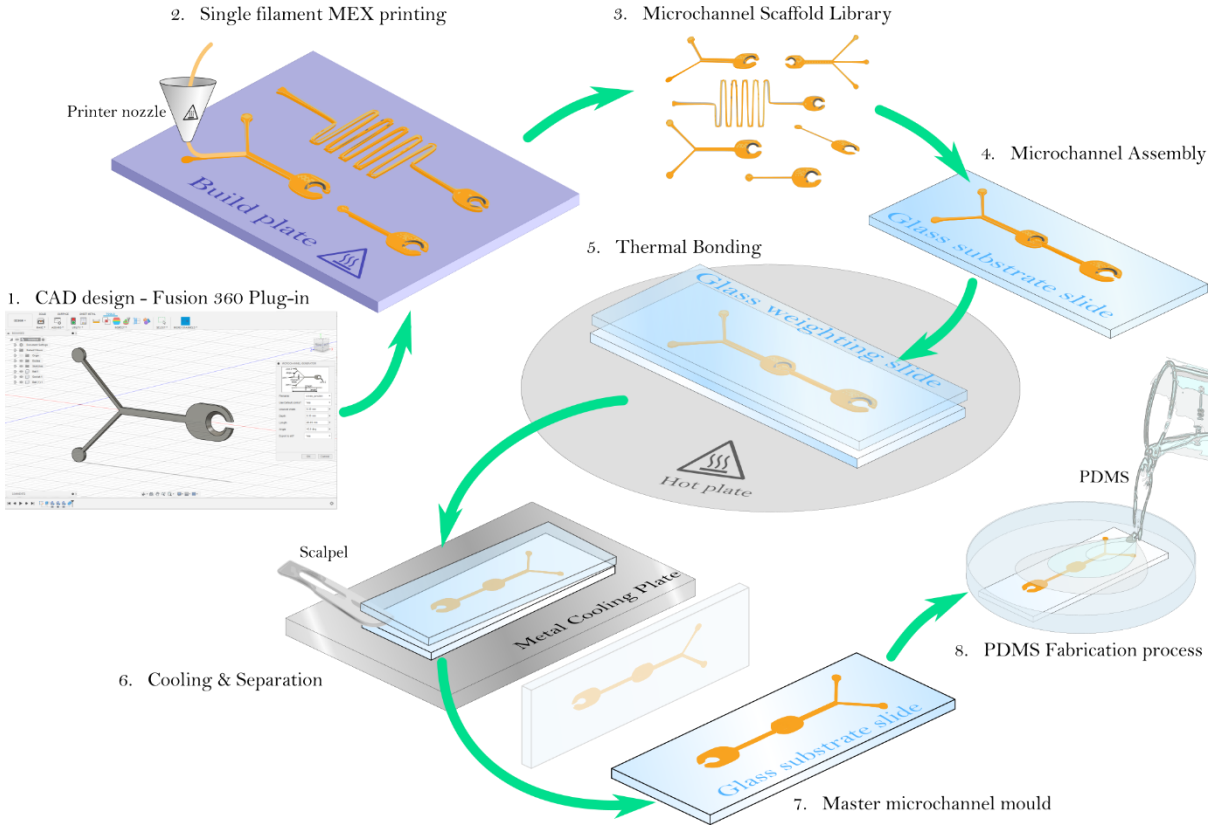


Figure 1 - Low-cost soft lithographic microchannel mould (negative) fabrication process. From [1].

3 Workshop Overview

This workshop will cover the low-cost soft lithographic steps required to produce a single microfluidic device in polydimethylsiloxane (PDMS) using the protocol described in references [1] and [2].

The workshop will guide the participants through the following steps shown in Figure 2:

Step 0: We begin with a library of pre-produced 3D-printed scaffolds,

Step 1: Assembly and thermal bonding to glass of the microchannel negative moulds.

Step 2: Soft lithography PDMS mixing, degassing, pouring and curing

Step 3: Separating the PDMS from the mould and assembling the microfluidic device on a glass slide with inlet and outlets

Step 4: Testing the channels for leaks and dye mixing (see Figure 3)

Figure 2 - The outcomes from each step of the manufacturing process in order. The numbering for each step corresponds to the appropriate section in this document.

Figure 3 - An example of a microfluidic device produced using this method performing mixing of a blue and a yellow dye.

4 Microchannel Mould Fabrication

4.1 Items Needed

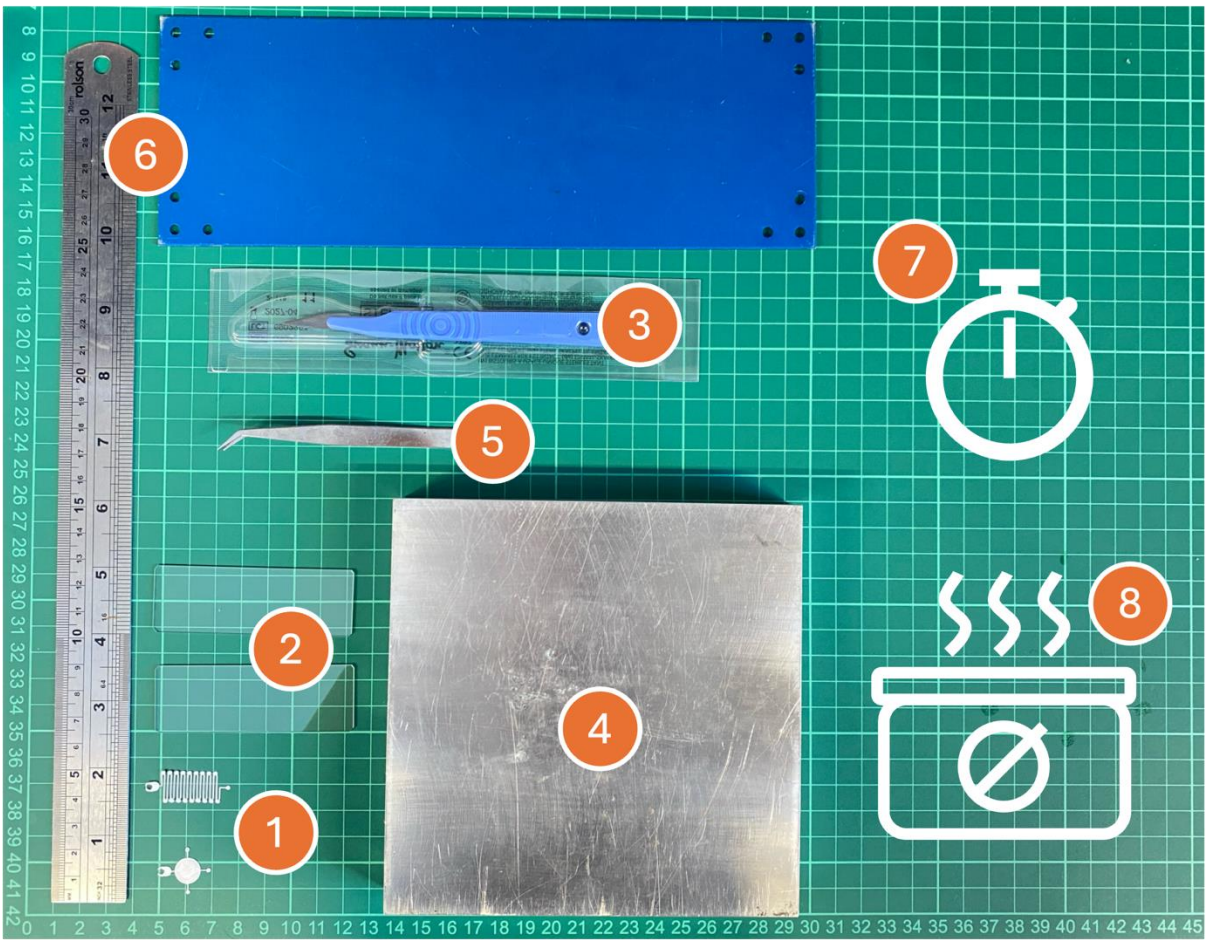


Figure 4 – Equipment needed for scaffold assembly and channel negative thermal casting

1. Selection of scaffolds
2. Standard Glass slides (at least 2) – 1mm thick
3. Scalpel or razor blade
4. Metal block – for cooling the slides rapidly
5. Metal tweezer – for moving scaffolds around and slides on and off the hot plate
6. Metal ruler or plate – For moving slides on and off the hot plate
7. A stopwatch or timer
8. Hotplate

4.2 Process

With reference to Figure 3 and youtube tutorial [3].

1. Heat up the hot plate (8) to 200 degrees C

2. Select a clean slide (2) for scaffold assembly (known as the *substrate slide*), and place it onto the metal plate (6)

3. Assemble the scaffolds (1) onto the *substrate slide*.

> **Top Tips 1:**
>
> *Try to place the scaffolds as far from the edge of the glass slide as possible. This will improve the seal of the PDMS to the glass slide and make cutting out the cured PDMS easier.*
>
> *Also note that the 3D printed scaffolds have two sides (a shiny side and a less shiny side). This asymmetry is a result of one side being in contact with the 3-d printer bed. The shinier side should be in contact with the substrate slide to improve bonding.*
>
> *Avoid touching the slide face or scaffold to try and minimise the debris on this mould to improve the quality of PDMS seal later.*

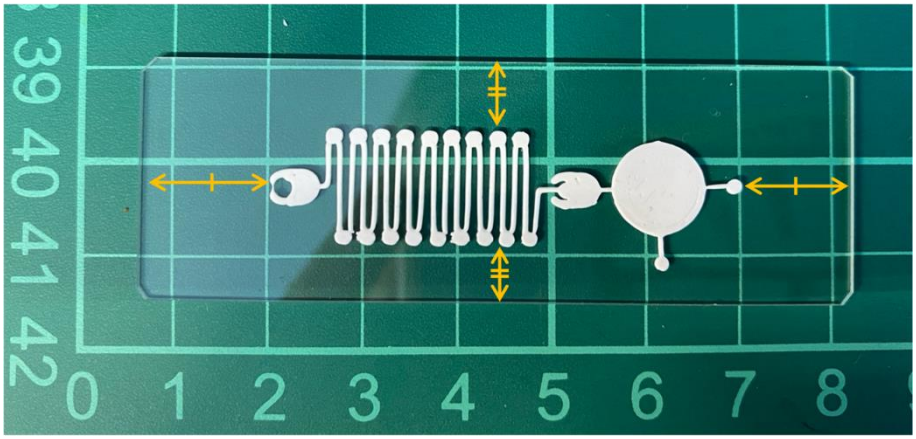


Figure 5 - A finished microchannel mould. Note that the scaffolds should be equidistant from the slide edges, and the bond between different scaffolds should be solid.

4. Apply a second glass slide, the *weighting slide*, on top. The weighting slide is there to apply even pressure across the scaffold during heating.

> **Top Tips:** It is okay to re-use *weighting slides,* however, we do not recommend re-using them as substrate slides.

5. Use the graph (Figure 6) to determine the optimum heating time based on the height of your channel scaffolds (for PLA)

> **Top Tip:** When timing the heating of the microchannel assembly note that underheating will result in poor adhesion to the glass substrate slide, and overheating will result in a wider, more squashed channel. Underheating can also cause issues at the connection points between scaffolds. If you think you have underheated the slide, it is okay to place it back on the hot plate with the weighting slide for additional time. See Section 0 on the impact of under and overheating.

6. Use the metal plate to carry the assembly to the hotplate, and use another glass slide or a metal ruler to slide the assembly onto the hot plate and begin the timer (30-45s) immediately.

> **Top Tips:** Take care when handling the microchannel assembly, particularly when placing the glass slide onto the hotplate. Small knocks at this stage can easily move the scaffolds which can result in poor alignment if connecting multiple scaffolds.
>
> We advise sliding the assembly off the metal plate by pushing on the bottom *Substrate Slide only* and avoid pushing on the weighting slide, taking care not to dislodge it as this can disrupt the uniform pressure that the weighting slide applies to the scaffolds.

7. Slide the thermally bonded assembly off the hotplate and onto the metal plate using tweezers, glass slide or metal ruler.

   Once off the hotplate, flip the assembly upside-down (*weighting slide* down) onto a metal plate or block to encourage rapid cooling on the weighting plate side.

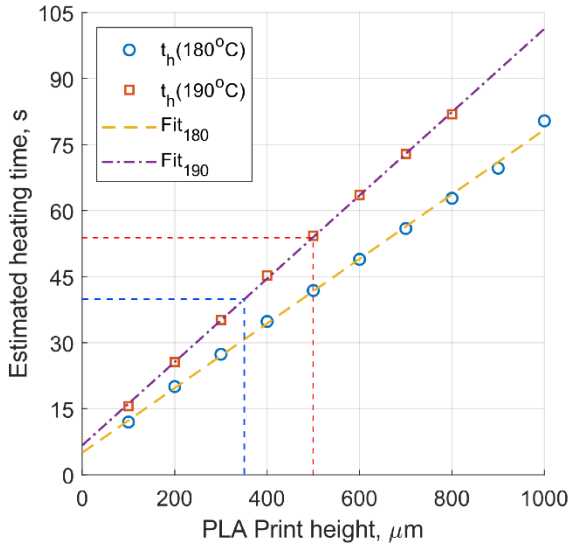


Figure 6: A graph showing the time it takes for the top of the scaffold to reach 180 or 190°C (melting point of PLA) for variable print heights, on a 200°C hot plate

8. Leave the assembly to cool until the top slide (substrate slide) is cool enough to touch comfortably (minimum 30 seconds)

> **Top Tip:** The slides should feel still warm but not hot as this makes separation easier than waiting for the slides to cool fully.

9. Use the scalpel to prise the slides apart. Slide the scalpel between he slides at a corner, and gently twist the blade. The weighting slide should detach easily leaving the substrate slide with the whole microchannel print stuck to it (*microchannel negative*) ready for PDMS pouring (see next section).

10. Place the *microchannel negative* into a petri dish to avoid contaminating the surface with dust. Do not touch the channel surface with your finger. Pick up the slide by the edges.

Top Tip:	Avoid touching the slide face or scaffold to try and minimise the debris on this mould to improve the quality of PDMS seal later.

4.3 Outcome

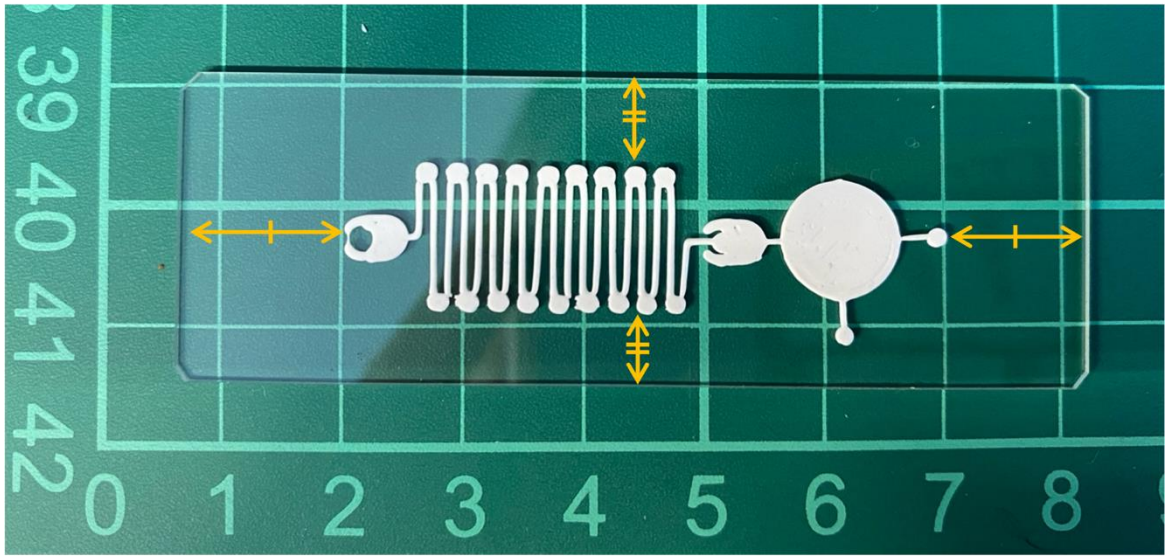


Figure 7: A finished microchannel mould. Note that the scaffolds should be equidistant from the slide edges, and the bond between different scaffolds should be solid.

4.4 Heating time effects

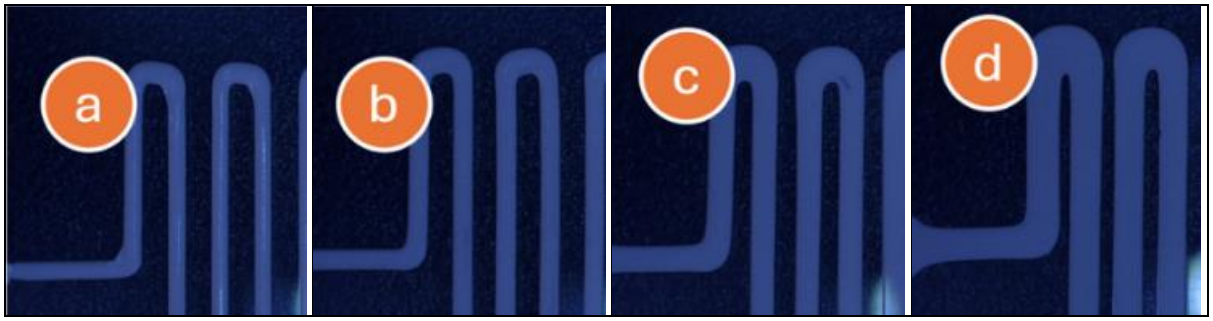
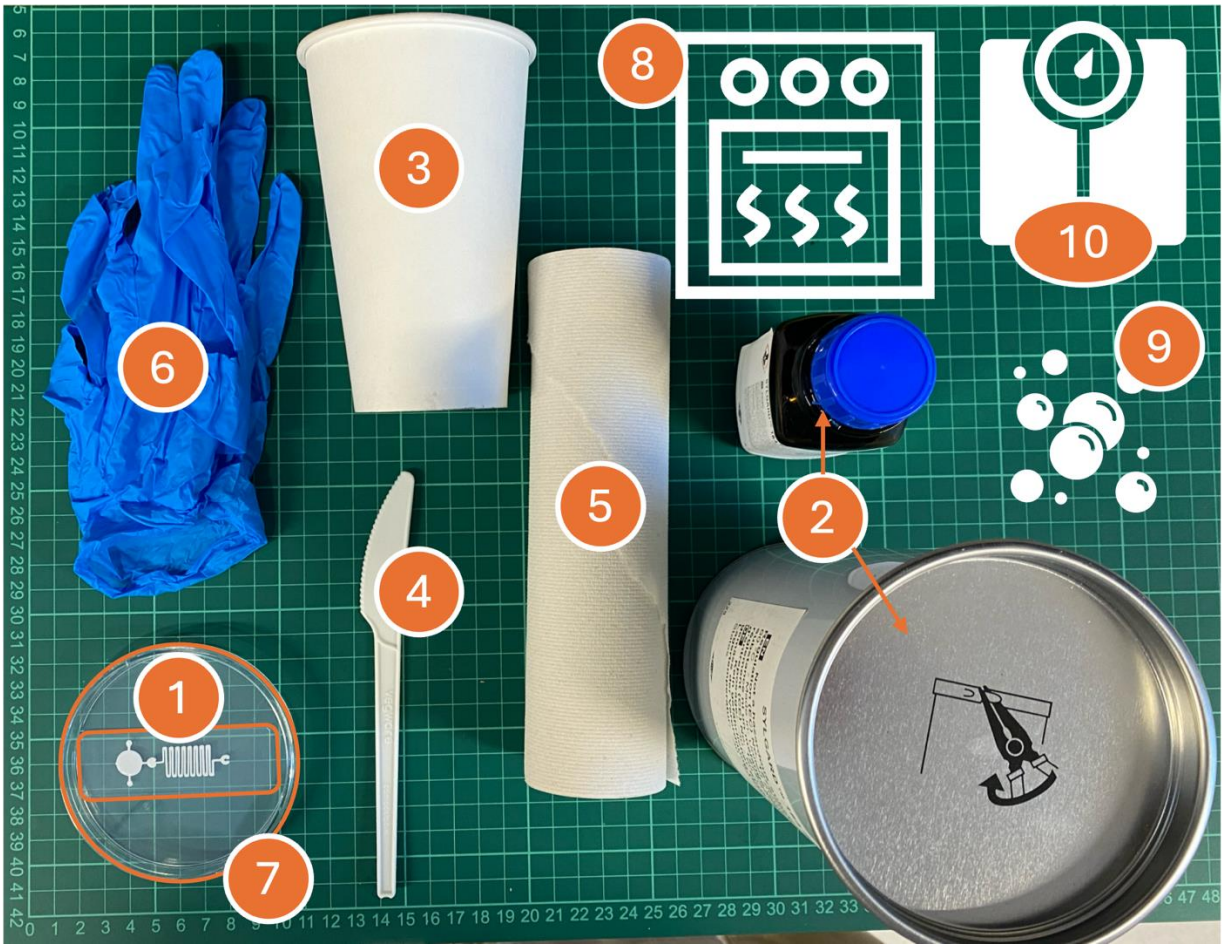


Figure 8: A figure showing the effect on scaffold dimensions for different heating times: a=15s, b=30s, c=45s, d=60s. These figures are for a hotplate at 190 degrees C and PLA of 0.3mm print height. This shows that 30s is close to optimal as dimensions only change a small amount.

5 PDMS Pouring

Risk assessment: RA 1986 for MMAT Lab

5.1 Items Needed



1. Microfluidic scaffold slide(s)
2. PDMS polymer kit (Sylgard 184) (PDMS and curing agent)
3. Disposable cup
4. Disposable stirrer – wooden stirrer, plastic spoon etc
5. Paper towels
6. Nitrile gloves
7. Tray or container for microfluidic scaffold slide – e.g petri dish
8. An oven set at between 40-70 degrees C
9. Vacuum degasser
10. Weighing scales

5.2 Process

1. In a disposable cup, weigh out and mix a 10:1 ratio of PDMS to curing agent in a disposable cup using a disposable stirrer – stir slowly to avoid too many bubbles.

> **Top tip:** Make sure whatever container you mix the PDMS in will fit inside the degasser you are using. If you are making many devices in one container, or using a large container, make sure to check that it is level while curing. Do not try and use water to measure the required volume of PDMS as water in the container can affect curing of the PDMS.

2. Degas the mixture in a vacuum degasser cycling the pressure. Do this until the bubbles stop.

3. In a suitable container for the microfluidic moulds, pour the PDMS over the glass slide and scaffold, and degas again to remove any more bubbles (see note 2 and 3)

> **Top Tips:** It can be useful to manufacture several devices at once. PDMS is one of the most expensive materials in this process and so it is desirable to minimise the amount that is wasted (i.e. any PDMS that is not on top of a glass slide). One way of achieving this is to line many slides up in a shallow dish (surgical trays can make suitable vessels).
>
> Make sure that the PDMS layer is thick enough such that any tubing inserted later can be inserted to a sufficient depth such that there are no leaks. We recommend aiming for the maximum depth of your biopsy punch (~5-10mm, see Section 3) on top of the slide. This allows for fluid channel adapters and tubing to be inserted more easily. Note that if the maximum depth of the biopsy punch is much less than the depth of the PDMS it can reduce the quality of the devices and cause leaks.
>
> A good way to pour the correct depth of PDMS is to mark it on the side of your chosen container before you begin pouring. If the sample is too thick (>10mm) then curing may take longer to cure.

4. Place into an oven at 40^{o}C – for overnight curing, or at 70^{o}C - curing in 3 hours.

5.3 Outcome

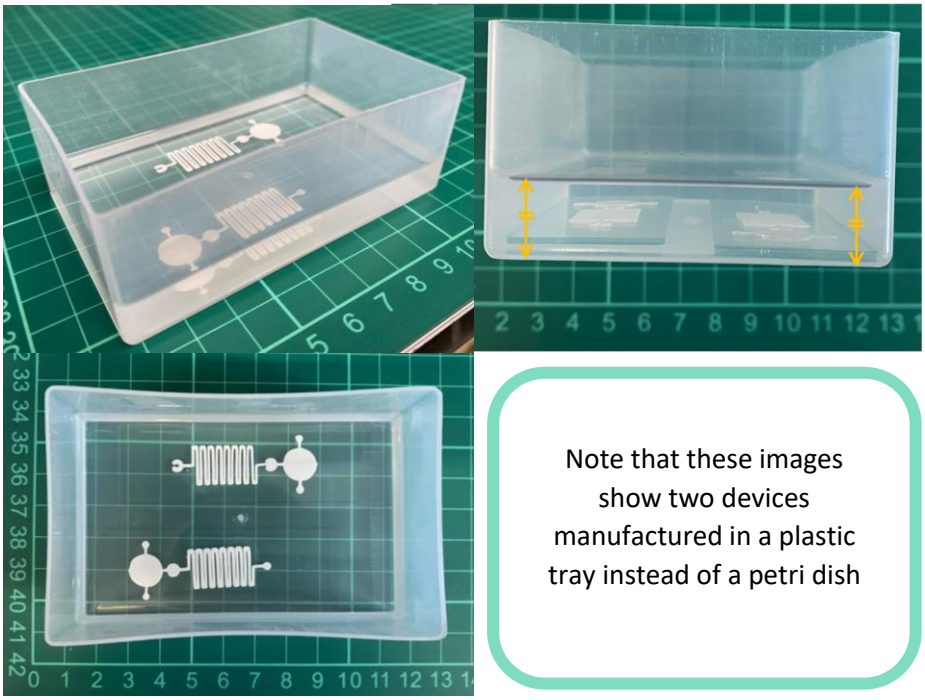
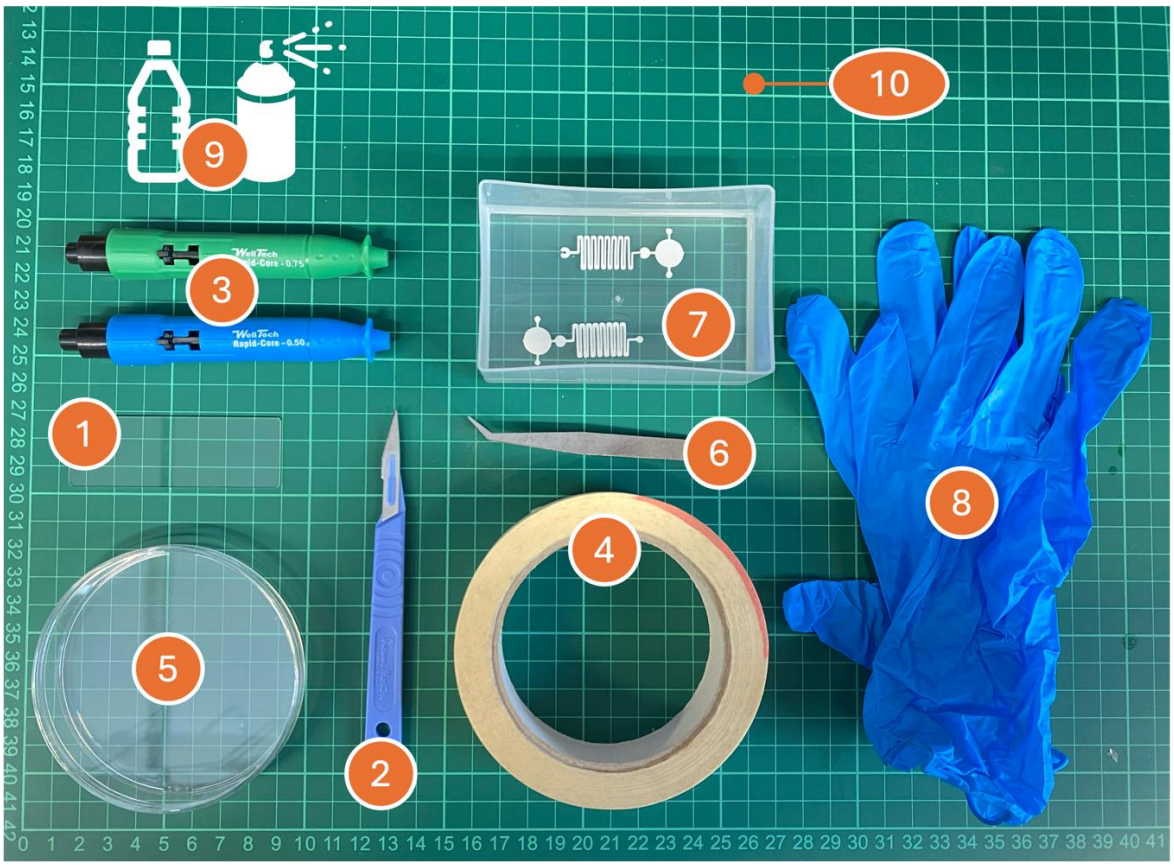


Figure 9: A figure showing the cured PDMS containing microchannel moulds. Note that the PDMS should be of equal thickness across the container.

6 Scaffold Removal and Finishing Steps

6.1 Items Needed



1. Glass slide (1 per device, unused)
2. Scalpel (new)
3. Biopsy punch – 0.5-0.75mm
4. Masking Tape (with a width greater than your glass slide)
5. Petri dish
6. Tweezers (very fine tip required)
7. Cured PDMS with scaffold
8. Nitrile gloves
9. Isopropanol alcohol and compressed gas spray can or air line
10. Cutting mat

6.2 Process

1. Cut some length of masking tape for covering devices before removing them from the PDMS to minimise time exposed to dust/air

2. Cut out the PDMS containing the scaffold using a scalpel and remove from container

> **Top Tip:** Be patient. At this stage of the process, it is hard to reverse mistakes since the PDMS is already cured. Hence, extra care should be taken. When cutting the PDMS with the scalpel use a sharp/fresh scalpel. If the edge of the PDMS tears it will likely damage the seal between the PDMS and the fresh glass slide, leading to leaking.
>
> Cutting too close to the channels can also lead to leaks, if there is not enough PDMS-Glass contact around the channel to prevent rupture of the device during operation. We recommend cutting out about ~2-5mm in from the edge of the slide.
>
> Avoid scraping the edge of the glass slide with the scalpel as this will damage it very quickly. You should also avoid sticking anything sharp (scalpel, tweezers) into the side of the PDMS to lever it out. If you cannot gently remove it with your fingers, cut an extra section out of the PDMS to enable your fingers to get underneath.

3. Sometimes the scaffolds can come away with the PDMS. This is fine. Use tweezers to remove scaffolds from mould.

> **Top Tip:** The microchannel negatives can be reused. If the scaffolds come away they can be re-bonded to a glass slide on a hot plate for a few seconds.

4. Use masking tape to cover the channel side of the PDMS, and keep it in a petri dish to avoid the contact surface collecting any dust.

> **Top tip:** It is the van de Waals bonds that maintain the adhesion between the PDMS and the fresh glass slide. Any dirt, dust, or debris that contaminates the interface between PDMS and slide can reduce the quality of the seal.

5. Use a biopsy punch on a cutting mat to make inlet and outlet holes in the channel mould

> **Top Tip:** Push biopsy punch down hard and slowly, once fully through the PDMS twist 180 degrees. Remove the punch and eject the inside of the hole. Take care lining up the punch and make sure the waste material is fully removed from the hole. Looking at the holes under a microscope can help.

6. If you fear you have contaminated the contact surface with dust or debris, you can use isopropanol and compressed air to wash and dry the contact surface.

7. Remove masking tape and apply the contact surface of the PDMS to a fresh glass slide and apply gentle pressure across the whole part

Top Tip: Here the goal is to remove any trapped air between the PDMS and the fresh slide that might reduce the seal quality. Corners are common problem areas, you can try to trim the corner if it will not adhere to the slide, however, be careful of going too close to the channels.

8. (Optional) Leave the device alone at this stage to promote better adhesion

Top Tip: We have noticed that if the device is left alone for several days the adhesion between PDMS and glass slide improves substantially. This is helpful in avoiding leaks during later testing.

We also recommend leaving the devices to heat for 1 hour at 80^{o}C with the glass slide in contact with the hotplate. Combining these methods can produce devices with impressive strength.

6.3 Outcome

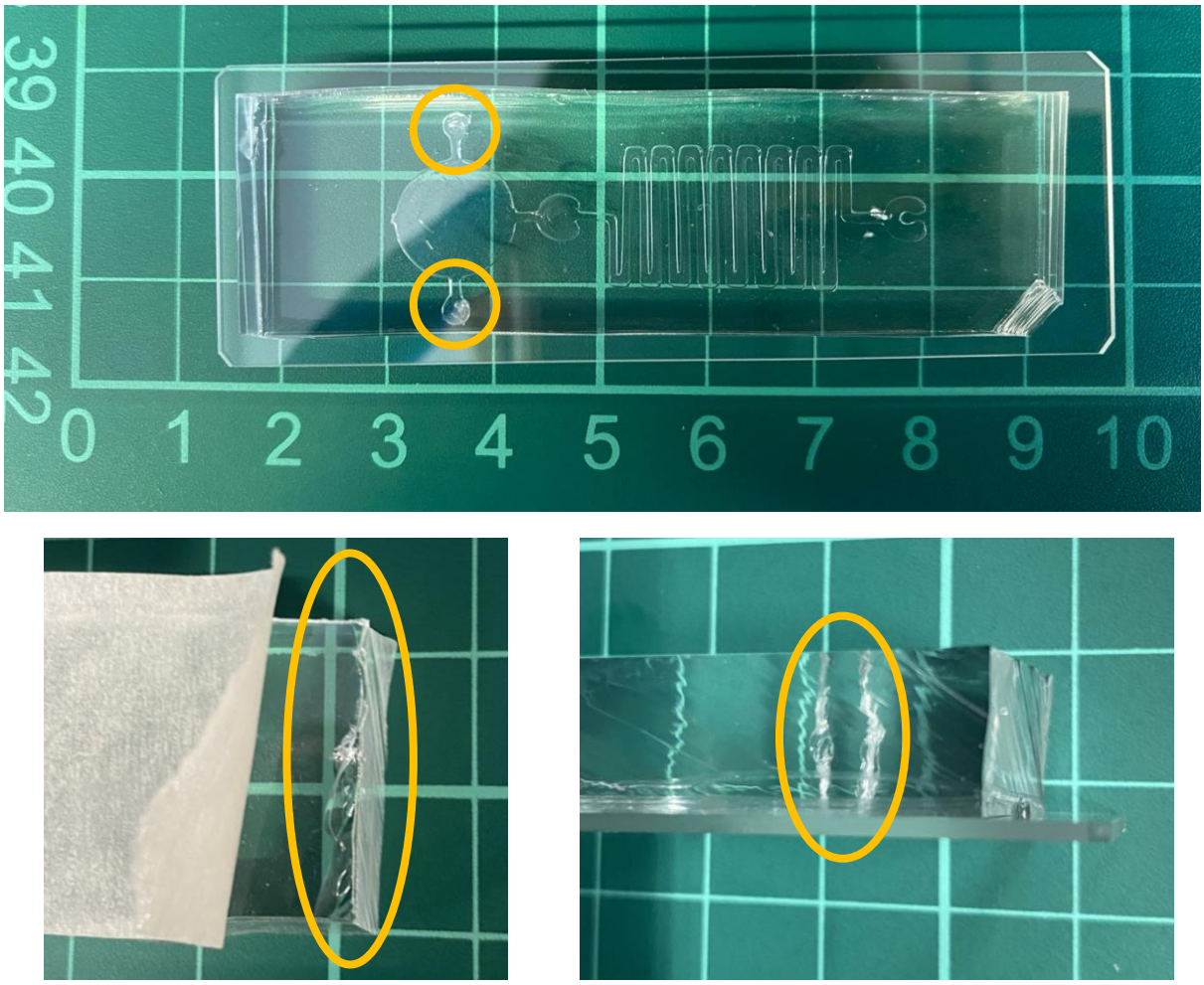
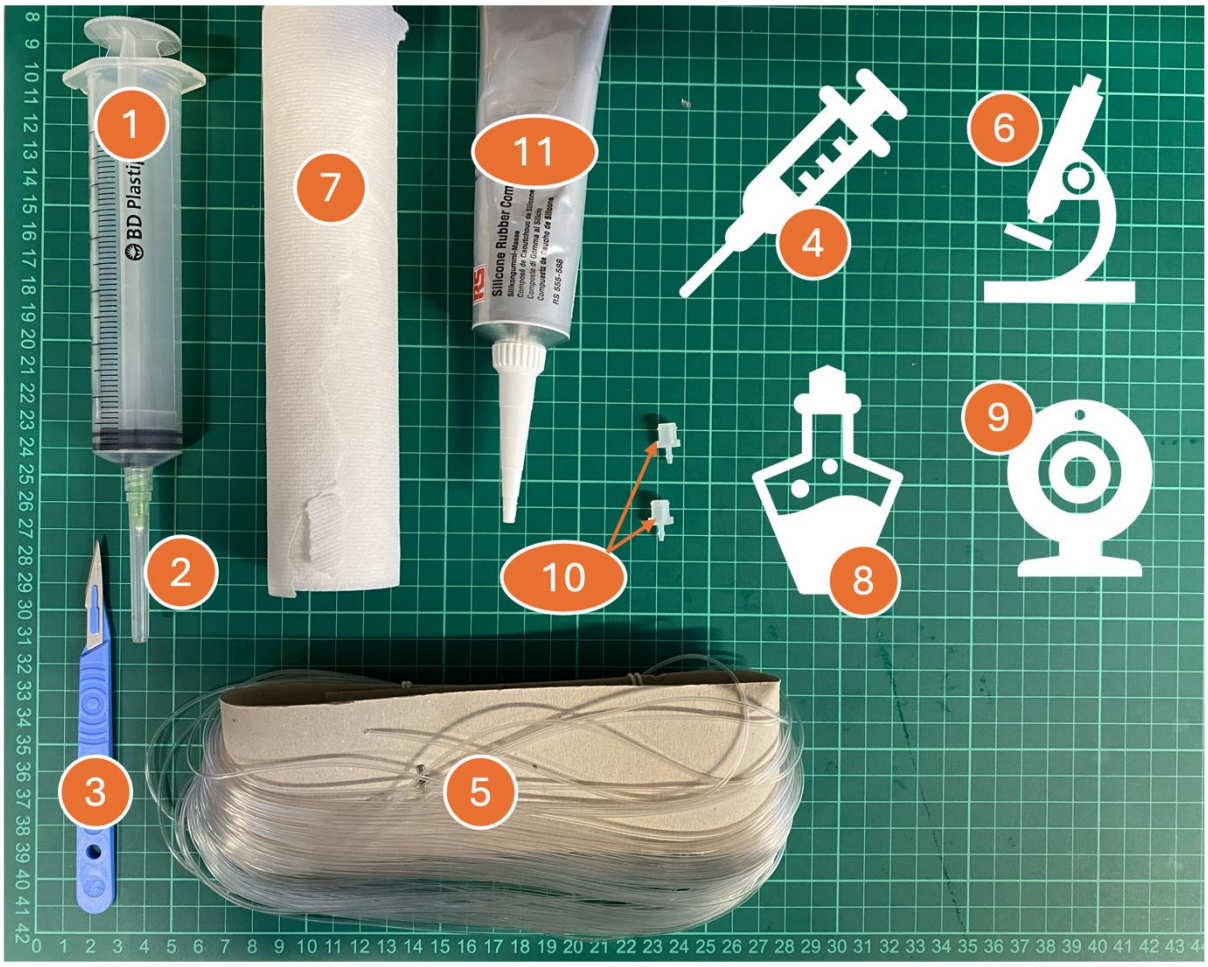


Figure 10: A collection of images showing the microfluidics devices near complete. These figures also highlight several possible faults. The top figure is a complete device, but the channels are too close to the edge of the PDMS to reliably avoid leaking. The bottom left figure shows a poorly cut PDMS slice, likely because of a damaged scalpel. The bottom right shows rough channels from poor biopsy punch use. Devices with these features are likely to leak.

7 Microfluidic Device Testing

7.1 Items Needed



1. Syringe
2. Syringe needles
3. Scalpel
4. Syringe pump
5. Microfluidic tubing
6. Microscope
7. Paper towels
8. Fluid / Dye

   Optional:

   9. Microscope camera
   10. Luer connectors
   11. Silicon glue

7.2 Process

1. Use the scalpel to cut a 45-degree angle on the microfluidic device end of the tubing

2. Insert the cut end of the tubing into the PDMS of the device inlet hole. Insert outlet tubing into the outlet hole in the same manner. The open end of the outlet tube should be flowing into a waste container/petri dish

> **Top Tip:** The tube insert sites are common areas for leakage so try to ensure the tube is inserted to around 50% of the depth of the PDMS without tearing the PDMS or damaging the tubing.

3. Position the device under a suitable microscope and focus onto the channel

4. Fill the syringe with the fluid of interest – i.e. dye

5. Using a syringe needle or Luer connector, attach a length of microfluidic tubing to the syringe and place the syringe in the syringe pump

6. Start the syringe pump (choose an appropriate flow rate setting) and observe what happens under the microscope

7.3 Outcome

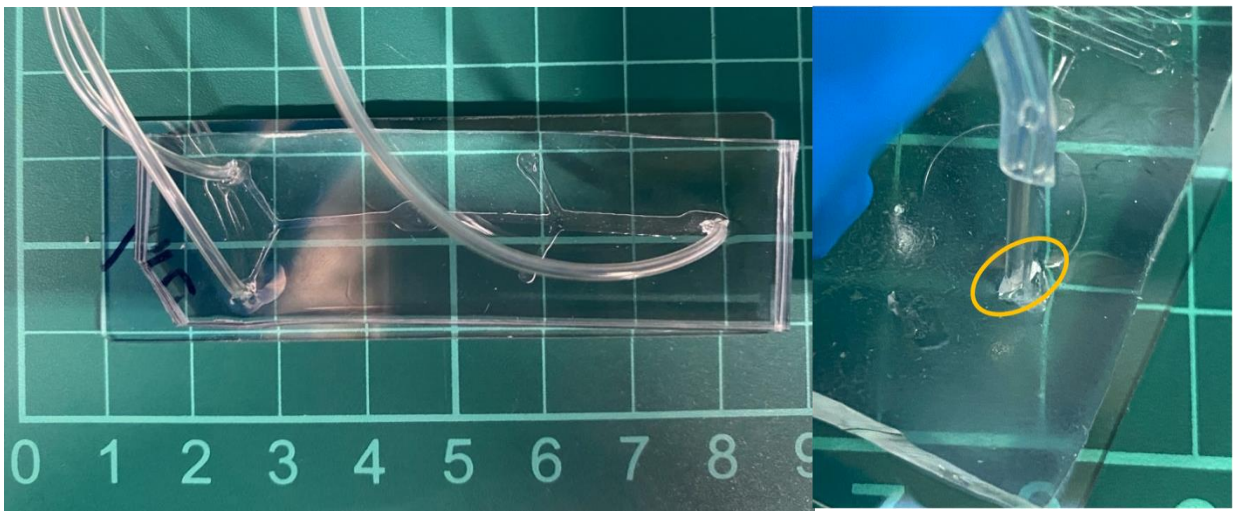


Figure 11: A figure (left) showing the microfluidic device complete with tubing inserted and a figure (right) showing a common issue when inserting tubing. The PDMS can split if tubing is not inserted carefully or if the PDMS is too thin. This is likely to cause leaks but can be fixed using silicone glue.