Microfluidic Chip Protocols

The creation of our microfluidic device started off by three dimensional designs in AutoCAD and/or Rhinoceros (see the Designs page). Those files were then converted to the file format .stl and put into the software called PreForm. That software is compatible with the Form 2, which was the 3D printer that we used. PreForm is also from where the printing process is started. In all our print jobs clear resin was used. From this point forward the printing process went as following:

- Remove mold carefully after printing is complete
- Wash the mold 2X 10 minutes in isopropanol (10 minutes in dirty isopropanol and 10 minutes in clean isopropanol)
- Treat the mold in an UV chamber for approximately 4 hours

The next step is making cured PDMS. Making cured PDMS is done by performing the following steps:

- Cover the working space with aluminum foil and put on gloves (preferably non-powdered ones)
- Pour uncured PDMS (9:10) and curing agent (1:10) into a Falcon tube
- Mix the content of the tube by inversion for 20–30 minutes
- Use the now cured PDMS immediately or store it in a -20 °C freezer
- Caution! The PDMS has to be well cured, otherwise the chip will not form properly

With the mold and cured PDMS ready the chip can now be assembled, which was done according to these steps:

- Tape (cardboard tape worked the best for us) the detachable wall together firmly with the rest of the wall (design dependent step)
- If frozen, thaw the PDMS
- Pour the PDMS into the mold up until a mark in the mold (include a mark to make pouring easier in your design if you want equally thick chips)
- Degas the PDMS in vacuum chamber and/or a refrigerator until all the bubbles are gone (we did 15 minutes in the vacuum chamber and 15–45 minutes in the refrigerator). The vacuum chamber we used was built by iGEM Uppsala 2016 (1).
- Bake the mold in an incubator or oven at 80 °C for 1–2 hours.
- Remove the tape and the detachable wall carefully from the rest of the mold.
- Use a scalpel to create a narrow gap between the mold and the chip so that it is possible to grip and separate the chip from the mold by hand
- Create the inlet and outlet with a needle
- Clean the mold using a scalpel and isopropanol

The chip is now almost ready to be connected to the rest of the experimental setup. That setup can, however, be prepared during the 1–2 hours incubation. The experimental setup contains the following items:

- Syringe needles (has to be compatible with the tubing; we used 23 Gauge needles)
- Puncher
- Tubing (many different sizes are needed as some need to be in the peristaltic pump, some connected to the needles and some in between the thicker and thinner tubing)
- Peristaltic pump

The setup was done by following these procedures:

- Separate the metallic and the plastic part of the syringe needles with the puncher
- Bend the needle to like a capital l.
- Calibrate the peristaltic pump to pump at the flow rate to have something being able to pass through the chip in 45 seconds (this must be calculated based on your design; in our case that flow rate was ~26 µL/min)

Now the chip can be covalently bonded to glass. To do that these actions were done:

- Clean a microscope slide and a plastic surface, for instance a plastic folder with isopropanol
- Place the microscope slide and the PDMS chip on the plastic surface and transfer it to the corona discharge gun.
- Set the gun to the highest setting and turn it on
- Sweep the corona discharge gun over the surfaces of the slide and chip that should be bonded for 30 seconds
- Press the treated surfaces together with some force
- Put the connected slide and chip in an oven at 80 °C for 30 minutes
- Let the chip cooldown for a bit and connect the L-shaped needles

Transformation can now be carried out on the chip. To do that the following material is needed:

- 70 % ethanol
- ddH_2O
- Plasmid
- $CaCl_2$ competent cells
- Media such as SOB or LB
- Agar plates with the right antibiotic
- Peltier connected to an Arduino and electricity source for the Arduino, such as a computer
- Pipettes and tips
- Heat block

- Ice
- Bunsen burner
- Matches
- Someway to spread the bacteria on plates such as a metallic loop
- Fully assembled chip

In the experiment, we need controls to be able to compare the efficiency of the transformation method, therefore experiment was divided into four separate parts:

- Perform regular heat shock transformation
- Add DNA but no heat shock is performed
- Heat shock without adding any DNA
- Heat shock through the chip with DNA added

The experiment was performed by performing the following steps:

- Put the chip on top of the Peltier to preheat it to 42 °C
- Preheat Eppendorf tubes with 950 μL media in them and plates at 37 °C
- Set the heat block to 42 °C
- Take out competent cells from the -80 °C freezer and thaw them on ice for 15 minutes
- Dilute your plasmid to an appropriate concentration (our initial concentration was 170 ng/μL it was diluted to have a concentration of 17 ng/μL)
- Once the cells had thawed they were transferred to Eppendorf tubes and 2 μL of DNA was added to all the tubes except the control without DNA
- Incubate the Cell-DNA mixture on ice for 30 minutes
- Pump some SOB into the tubing and then air to later be able to track the transformation mixture through the tubing (it does not need to reach the chip)
- After incubation, load one tube of cells into the chip system (the flow rate remained the same throughout the experiment), perform heat shock on the heat block for 45 seconds on the labeled with regular heat shock. The last tube was kept on ice
- After heat shock on the heat block incubate the cells on ice for 5 minutes
- After incubation, transfer the cells to the tubes with preheated media and keep them at 37 °C for 1 hour and invert the tubes a few times during that hour
- Collect the cells from the chip into media and then pipette 250 μL of the cell-media mix into four new Eppendorf tubes, then incubate them at 37 °C for an hour and invert them a few times during that hour
- Pipette 100 μL of each sample on separate plates and spread bacteria with the metallic loop (keep a lit Bunsen burner and ethanol close by to sterilize the loop)
- Incubate the plates at 37 °C overnight

Chip had to be cleaned after a run which was done by pumping 70 % ethanol through the system, then ddH_2O and lastly some air. The chip had to rest for at least 30 minutes after the cleaning process before it could be used again.

References

1.Team:Uppsala/HP/Gold - 2016.igem.org [Internet]. [cited 2017 Sep 28]. Available from: http://2016.igem.org/Team:Uppsala/HP/Gold