Lipodrop 2.0 device coating

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Aim:

To selectively coat channels in order to make them hydrophobic (pre junction channels) or hydrophilic (post junction channels).

Reagents used:

Table 1. List of reagents used in the experiment

Name of reagent	**Cat. No.**	**Lot number** *(if applicable)*
Polyvinyl alcohol (PVA)		

Table 2. List of tools used in the experiment

Name of a tool	**Quantity**	
3 mL plastic syringe	2	
1 mL glass syringe	1	
Syringe needle	3	
Tubing		
Tweezers		
Razor blade		

Experiment description:

This protocol aims to render the post-junction part of the device hydrophilic. This is done in order to prevent the lipid/octanol phase from wetting the inherently hydrophobic surface of the device. The hydrophilic treatment is achieved by injecting a 2.5% (wt/vol) 67 kDa polyvinyl alcohol (PVA) solution through the OA channels and letting the PVA molecules adhere to the surface. It is critical for a successful liposome synthesis.

EXPERIMENT PROTOCOL:

1. Prior preparations

a) PVA

In order to prepare 2.5% (wt/vol) solution weigh 1.25g of PVA powder. Pour 50 mL of water into glass vial and put it on magnetic stirrer with heating function. Turn the magnetic stirrer on to vigorously mix the solution (600rpm) and raise the temperature to 80°C after that slowly pour PVA powder into the water (You should pour whole powder in 5 minutes Important: it is a crucial step to avoid PVA clumps in the solution). After you dissolve PVA in water, there should be no clumps left in it and the solution must be clear.

Note: *You should keep in mind that PVA dissolves slowly (more than 4 hours). In order to make precise concentration you should cover the vial while stirring the solution to avoid water evaporation.*

As an alternative to that preparation, I would suggest to dissolve it a little bit and then bring it to autoclave. It makes PVA to dissolve much faster.

Note: *Even though I followed more than one protocol, I did not manage to avoid small clumps. While it is really important to have dust-free and clumps-free solution, I tried to filter it with 400 nm filter, however, the solution is too viscous and it stuck in the filter. Later on, I strained it with 70µm cells strainer and it worked fine. Skip this step if you have enough of stored PVA.*

b) Microfluidic Devices

Cut the tape on top of the PDMS with a razor blade between separate microfluidic devices for an easy access later.

c) Syringes

Prepare syringes for air *(3 mL plastic syringes):* secure the needles on the syringes and put the tubing on with the tweezers.

Prepare a syringe for PVA: fill the syringe with PVA (use a pipette: gently touch filled pipette tip to the syringe opening and pull down the syringe grasp; make sure to avoid any bubbling) and put on the needle and then the tubing. Carefully press the syringe grasp up until devoid of air. Place the syringes vertically in appropriate places on the pumping device. Make sure to secure them firmly on with all of the holders (3 separate ones). See ***Fig. 1*** for details.

Note*: the tubing should be appropriate length (enough to reach the channels but not wasteful).*

Note*: check the air syringes before every coating procedure: the plunger of the syringe should be pulled back.*

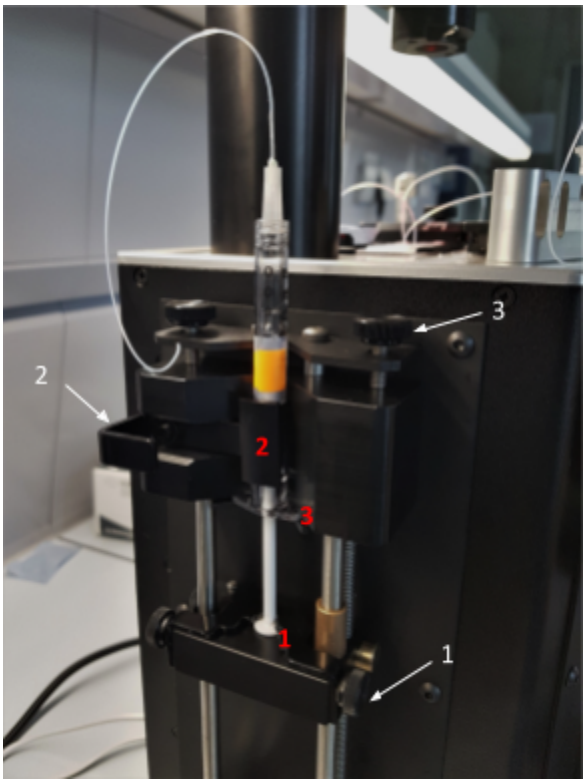

Fig 1. Syringe placement setup
Red numbers indicate where the syringes should be secured, while white numbers mark appropriate screws for fastening the syringes in place.

d) Connecting the DropGen portable microfluidics station

a) Turn on the station by plugging it to the electrical socket. Simultaneously, turn on the computer and connect to DropGen Wi-Fi. Then visit the website: *192.168.1.100*

b) For this experiment a default 3 syringe interface can be used. There is an option to name the pumps by selecting **Pump settings** in the **Settings** section found on the same page.

c) Camera view window should appear on the left part of the website (if not, restart the device or the webpage, or both).

d) To focus the camera, use the silver knob on the pumping device. For quick adjustment press ***Auto adjust*** on the webpage right below the camera view. It is possible to adjust illumination, exposure and FPS manually.

e) Pumping velocities can be regulated on the right panel of the website.

e) Syringe priming

To fill the tubes with material and make sure that syringes are firmly placed on the device **press purge** until you see the material (PVA) visibly at the end of the tube. (this should be done to air syringes as well).

Before work, make sure that the portable station is clean from any dust as it potentially could clog the channels of devices. Prepare wipes for collecting the waste (they should be right under the waste tubing).

2. Microfluidic device scheme

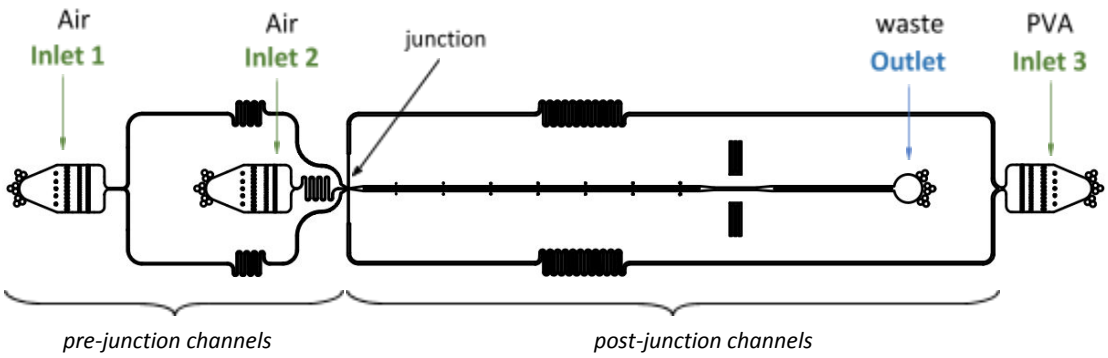

Fig. 2. Scheme of Lipodrop 2.0 microfluidic device. Green arrows indicate different inlets while blue arrow marks the outlet.

3. The coating procedure

Important: *The coating procedure is only a rough guideline for the coating process. One should consider that each microfluidic device is slightly different (channel height, proportions, etc.), some of which might have impurities or defects that would require adjustments to the suggested velocities. Keep in mind that the process is dynamic and requires continuous attention. One of the most important parts of the process is achieving a correct and stable meniscus at the junction (See* ***Fig.3****)!*

a) Put the tubing connected to the air syringes into **Inlet 1** and **Inlet 2**. Set the flow rates to 1000 µL/h and turn the appropriate pumps on. Wait half a minute before putting in the PVA tubing.

Note*: air should be the first one turned on and the last one turned off.*

b) Put in the waste tubing to the **Outlet**.

c) Put in the PVA tubing to the remaining hole. Wait at least 1 minute before turning PVA on.

d) Turn the PVA on with the flow rate between 50 µL/h and 150 µL/h and wait for the PVA to appear. Once it flows into the inlet, turn it off ASAP and increase the flow rates of air to 1500 µL/h.

e) Observe the situation at the junction. If everything is correct, the PVA and air should meet at the junction **without PVA entering pre-junction channels.** There is a chance that it won't reach the junction while turned off. In that case turn on the PVA (lower than 50 µL/h) and gradually decrease the air velocity.

Note: *If PVA does infiltrate pre-junction channels, discard the device and start over.*

f) After a couple of tens of seconds (needed for the pressure to settle) set the PVA to 50 µL/h and turn it on (if turned on before, increase it up to 50 µL/h).

g) The aim is to get a junction looking like ***Fig. 3*** To reach it, adjust flow rates of air and PVA. Usually, once PVA has travelled to the junction and closed it, there is sufficient pressure in the pre-junction channels to deflect the air, therefore it can be gradually decreased according to the situation. (It could even go down to 100 µL/h in some cases). At the same time PVA is increased (up to 600 µL/h in some cases).

Note: some air bubbles leaving the pre-junction channels

h) Keep a steady meniscus at the junction for 4-6 minutes.

i) Take out the PVA tubing (while on) and then turn it off.

Note: *before taking out the tube, make sure that the post-junction channel is devoid of bubbles, as it creates unwanted bumps while PVA dries.*

f) Increase air flow up to 1500 µL/h to dry out the remains of PVA.

g) Turn off remaining pumps and take out the tubing.

h) Before putting back on the tape, blow out any remains of PVA with a syringe (without any needles). If the coating was correct, mark it as such with a marker.

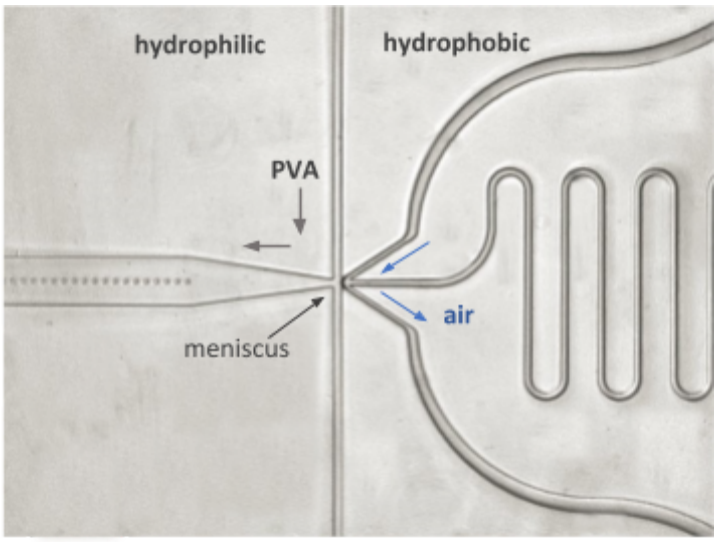

Fig. 3. A correct meniscus at the junction during the coating process. Grey arrows indicate the direction of the PVA while blue arrows indicate direction of air. Meniscus is observed at the junction.

ADDITIONAL OBSERVATIONS AND IDEAS:

Possible mistakes & troubleshooting

- Always be observing the PVA inlet during the injection as it can appear quicker than anticipated.
- Make sure to check the correct arrangement of the microfluidic device before putting in the tubing.
- Check for the defects before every measurement to avoid wasting time and reagents on a defective device. Defects might include dust particles, closed channels, detached PDMS, discontinuous channels etc.
- If the PVA is not appearing, check if the tubing is not leaking or if it is not primed correctly (i.e. with bubbles). In that case change the tubing or purge the PVA, accordingly.