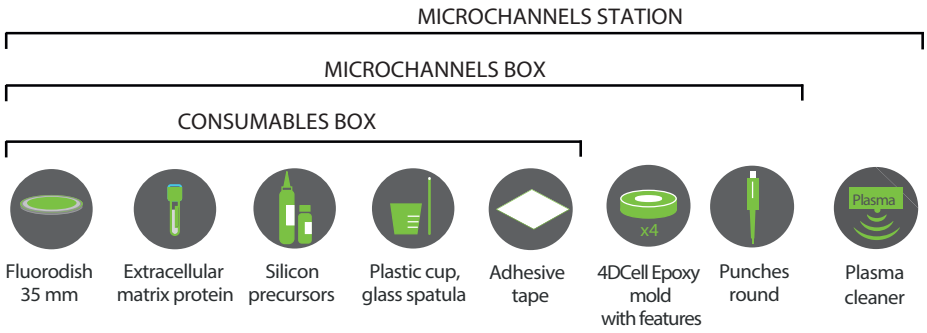
MICROCHANNELS KIT
on PDMS chip with epoxy mold

Material included



Extra material recommended not included in the kit

Vacuum jar bell
Oven (up to 80°C)
Micropipette 100-1000 µL
Micropipette 2-20 µL
PBS solution

For the PDMS precursors, the weight ratio ELASTOMER:CURING AGENT is 10:1 and the volume ratio is 12:1.

A. MOLDING OF THE CHIP

1. Fill the graduated plastic cup with the curing agent and the PDMS elastomer at a ratio of 1:10 in weight.
   Ex.: for 1 chip, mix 3 g of the 4Dcell PDMS elastomer with 0,3 g of curing agent. Chips should be about 5 mm height maximum.
2. Homogenize thoroughly the mix for 3 minutes using the glass spatula.
3. Remove trapped bubbles by degassing the mix with the vacuum jar bell for about 45 minutes.
   Alternatively, centrifuging for 10 minutes is also possible.
4. Pour the PDMS into the epoxy mold(1).
5. Degas once again (for about 5 minutes), until there is no bubbles left.
6. Place the mold in the oven at 80°C for 1 hour(2).
7. Once cooled down, gently unmold the chip in alignment with the microchannels.
8. Place the chip on a clean and flat surface, channels facing upwards. Using the round punch, cut 3 mm diameter holes at each access port of the channels.
9. Before proceeding to the next step, remove residual dust from the dish using lens cleaning paper and clean the chips by delicately applying adhesive tape(3).
   This will promote the binding of PDMS to glass.
10. Activate the PDMS (structures upwards) and culture dishes by air (or oxygen) plasma treatment during 2 min at 300 mTorr.
11. Place both activated surfaces in contact to permanently stick the PDMS to the substrate. If needed, use metallic forceps to press slightly on top of the PDMS to force the contact between the polymer and the glass of the dish. Avoid to press directly on the microchannel patterns to avoid PDMS collapsing.
12. Incubate the chip in the oven at 65 °C for 1 hour to strengthen the binding.

B. ADHESIVE SOLUTION COATING

1. Reactivate your chip and the Fluorodish in the plasma cleaner chamber (2 min, 300 mTorr).
2. Directly fill the channels with 10 µL of surface coating solution(4) per access port.
3. Incubate one hour at room temperature to allow adsorption of the coating substrate(5).

(1) To clean the molds without solvents, molding a chip once before will allow to stick dusts to the PDMS.
(2) If you don't have an oven, you can still let the mold at room temperature and wait for the day.
(3) Avoid touching the chips on the microchannels side not to deform microstructures, nor preventing chip bonding to glass.
(4) 4Dcell adhesive coating solution is based on fibronectin at 10 µg/mL. The concentration should be adapted depending on the cell type used and the results expected. Other substrates such as collagen, PEG, ... may be used to modify cell adherence to the channel walls.
(5) The solution should infiltrate the channels by capillary forces: make sure that the liquid spreads throughout the entire structure. This can be easily checked by eye under regular light or using a regular bright field microscope. In very small structures, in which diffusion is harder, entry of liquid in the channels can be forced by placing the structure in a vacuum jar bell during at least 15 min.

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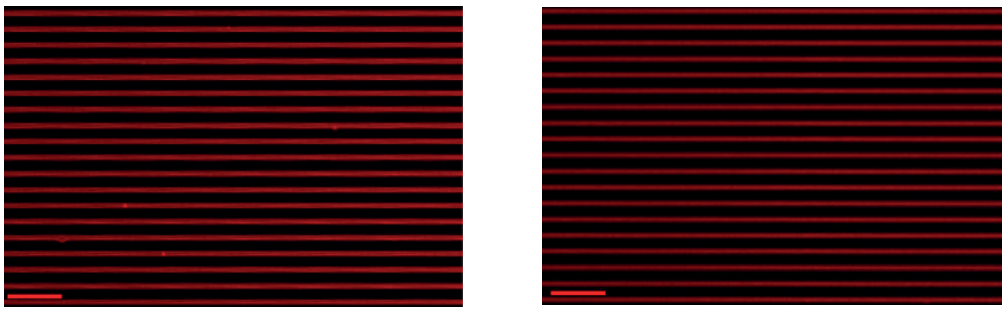
C. COATING TESTING (optional)

To evaluate the efficiency of intra-channel coating, fluorescent or colored substrates can be used. In this case, the coating solution should be used at 100 µg/mL and the channels washed with PBS after coating. The remaining fluorescence can be directly observed under an appropriate microscope.



Coating of channels with a fluorescent substrate.
Left image: 5 µm x 5 µm channels incubated with fluorescent PEG at 100 µg/mL during 1 hour
Right image: Image representative of the remaining fluorescent PEG after PBS washing of the channels. Bar 50 µm.
Study of cell migration in microfabricated channels. Vargas P., Terriac E., Lennon-Duménil AM., Piel M.. J Vis Exp. 2014 Feb 21;(84):e51099. doi: 10.3791/51099

D. PLATING THE CELLS

1. After the fibronectin coating, Wash the dishes by immerging the PDMS in 3 mL of PBS. Remove it either with an aspirating pump or by flipping the dish. Ensure not to aspirate directly inside the chambers to prevent from drying.
2. Repeat this step twice but let the PBS incubate for 5 minutes before removing it.
3. Similarly, rinse 3 times the channels by immerging them into 2 mL of pre-heated culture medium(6)(7). Incubate the device with 3 mL of medium at 37°C for 15 minutes(8).
4. Aspirate the medium and discard 10 µL of solution from each chamber.
5. Place a droplet of cell solution (5 µL or 10 µL) per access port. Cell concentration should be adjusted to have a confluency of 60-70% inside the hole. To achieve this, we advise a cell concentration ranging from 10^6 to 10^7 cells/mL(9).
6. Close the lid of the Fluorodish. Place the Fluorodish into a humidified incubator with the appropriate settings for the cell type used (typically for mammalian cells this will be 37°C and 5% CO_2) to allow cell adherence. Incubate at 37°C for 30 minutes to one hour.
7. Add 2 mL of culture medium(6).
8. Incubate at 37°C, 5% CO_2 at least 4 hours(10) before observing the cells into the channels.

(6) The whole PDMS structure must be covered. If 2 mL is not enough, add more medium to completely cover it.

(7) For experiments involving drugs like molecule inhibitors, it is advised to preincubate the channels with a medium containing the drug at the right concentration.

(8) The PDMS absorbs molecules in the medium. So if you have fragile cells, an overnight incubation with PBS (3 mL) at 37°C is recommended before seeding the cells.

(9) High cell density is required to stimulate the contact of the cells with the channels. Low cell density may result in low number of cells inside channels and failure of the experiment. If needed, apply an "up & down" movement with the pipette to push the cells towards the borders.
(10) This duration will depend on the cell characteristics and migratory capacities.

Reference: Vargas P., Terriac E., Lennon-Duménil AM., Piel M., "Study of cell migration in microfabricated channels", J Vis Exp. 2014 Feb 21;(84):e51099. DOI: 10.3791/51099.
Vargas P., Chabaud M., Thiam HR., Lankar D., Piel M., Lennon-Dumenil AM., "Study of dendritic cell migration using micro-fabrication", J Immunol Methods. 2016 May;432:30-4. doi: 10.1016/j.jim.2015.12.005. Epub 2015 Dec 9.
Vargase P., Saez P., Barbier L., Attia R., Thiam H., Piel M., "Leukocyte migration and deformation in collagen gels and microfabricated constrictions", in Cell Migration: methods and protocols, Methods in Molecular Biology 1749 edited by Alexis Gautreau, Laboratory of Biochemistry, Ecole Polytechnique, Palaiseau, France, 2018, pp. 368-369

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