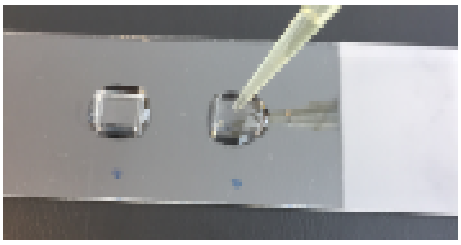
# Membwell Differential Coating Protocole

## I. Coating of the side wall of the Membwell micro-wells:

1. (Optional) Plasma treatment of Membwell for 3-5 min.

2. Put proteins solution on Membwell, to coat the sides of the micro-wells.



3. Vacuum for 5 mins to allow the protein solution to enter into the micro-wells

4. Incubate for 1h at room temperature.

## II. Coating of the bottom of the micro-wells:

1. In parallel, coat a coverslip, or glass-bottom dish with the proteins of your choice. This protein will be at the bottom of the micro-wells.

2. Incubate the coverslip with the protein solution for 1h at room temperature.

3. Note: The protein concentration and coating duration depends on protein type. Here are some indications:
    - Fibronectin/ BSA: 15ug/ml, 1h in room temperature
    - E-cadherin: 30ug/ml, 4°C overnight
    - Protein A conjugated (to visualize E-Cadherin): 50ug/ml, 1h

## III. Flipping of the Membwell onto the coated coverslip:

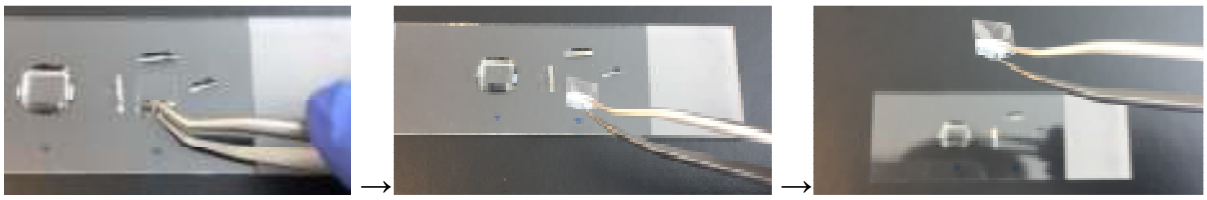
1. Rinse the Membwell membrane and glass-bottom dish with water for 3 times.
2. Aspirate the water and allow the membrane and glass-bottom dish to dry completely.

**Important Notes:**

- You need to flip the Membwell membrane in order to avoid non-specific adhesion at the top of micro-wells.
- To have a good adhesion between the Membwell and the coated coverslip, be sure that the coverslip is completely dry. The Membwell also needs to be dried, but not completely. Just standard aspiration will do.

3. Hold on to the edges and push them away from the membrane region, only leaving one edge (marked by the **Blue** dot).

4. Peel off the membrane by grabbing the edge left.



5. Flip the membrane onto the coated coverslip.

6. Cut the last edge with a scalpel.

7. To ensure full contact between the Membwell membrane and the coverslip, a flat and clean Parafilm can be used to press gently on the membrane.

## IV. Passivation of the top of the membrane to avoid non-desired cell adhesion out of the micro-wells:

**Important Note: Please be very gentle while you refresh, top-up, aspirate medium/liquid in the Membwell dish, otherwise the Membwell membrane may detach from the coverslip.**

1. Passivate the top of the flipped Membwell membrane with 0.2% pluronic acid (Sigma, Pluronic-F127) diluted in PBS for 30min.
2. Rinse the Membwell membrane with water for 3 times.
3. Add sterile PBS and vacuum it for 5 minutes to fill the micro-wells with PBS.

Note: The Membwell membrane can be kept overnight at room temperature sealed with Parafilm.

## V. Cell seeding:

In biosafety cabinet:

1. Wash the Membwell once with sterile PBS.
2. UV treatment for sterilization for ≥15min.
3. Exchange PBS with medium and incubate in 37°C until use.

The following protocol depend on the cell type and micro-wells size:

4. Remove the media without drying the micro-wells by leaving a small amount of media around the micro-wells membrane.
5. Seed about 0.1-0.5 million cells and incubate for 30min to 1hr to let the cells fall and adhere into the micro-wells.
6. Wash with warm media and incubate the cells for another 15 to 30min.
7. If the cells density is insufficient, seed again with 0.1-0.5 million cells and repeat the previous steps.

**Important Note: Please be very gentle while you refresh, top-up, aspirate medium/liquid in the Membwell dish, otherwise the Membwell membrane may detach from the coverslip.**