1. Objective

Seed cells in a previously wetted channel of the OrganoPlate® (e.g. after channel coating or for tube-in-tube seeding, in which a second cell type is added to grow within a tubule of already existing cells).

2. Background

The underlying principle (illustrated in an OrganoPlate® 3-lane in Fig. 1) is that the surface tension of a droplet of 1-2 µL is higher than that of 20-100 µL of medium, thereby causing the droplet to retract and the cell suspension to be pulled in. As the channel is still pinned on one side, flow is suspended, and the cells can attach.

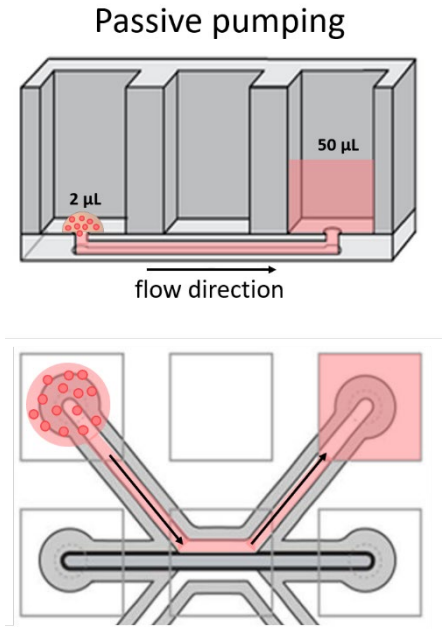

Figure 1. *Illustration of underlying principle of passive pumping seeding technique in an OrganoPlate® 3-lane.*

3. Materials

- 3-lane 400µm OrganoPlate® (MIMETAS, 4003-400-B)
- Repeating pipette for cell seeding. We recommend the Eppendorf® Multipette® M4 with the Eppendorf® Biopur® 0.1 mL tip (VWR #613-2067) or the Sartorius eLINE® electronic pipette (Sartorius, #735021) with corresponding Sartorius tips or with Eppendorf® ep Dualfilter tips (Eppendorf, 022491211 / 0030077512)
- Multichannel pipette (1200 µL or 300 µL)
- Multichannel tips
- Cell suspension (at desired concentration)
- Medium

4. Procedure

1. Aspirate liquid from all wells (excluding observation windows)
    a. Make sure to thoroughly aspirate to avoid low amounts of liquid persisting around the inlets and outlets
2. If the **inlets** of the desired channels have been cultured with medium, they need to be washed once with PBS (see steps below). If not, proceed to step 3.
    a. Dispense 50 µL PBS on the **inlets** of the channel to be seeded
    b. Aspirate dispensed PBS thoroughly
3. Dispense 50 µL medium on **outlets** of channel to be seeded

a. Do not add medium to inlets or outlets of other channels for now. This could introduce an adverse flow of liquid and/or cells during attachment

4. Dispense 1-2 µL cell suspension on **inlets** of the same channel
    a. Check under the microscope whether the cell suspension has entered the channel. This step can potentially be repeated if initial seeding is not satisfactory by adding another 1-2 µL droplet
5. Place the OrganoPlate® on its side in the MIMETAS plate stand (see Fig. 2) or flat in the incubator until cells have adhered
6. Dispense 50 µL medium on **inlets** of seeded channels
    a. If desired, medium can now also be added to other channels (e.g. bottom channel perfusion for Caco-2)
7. Start culture

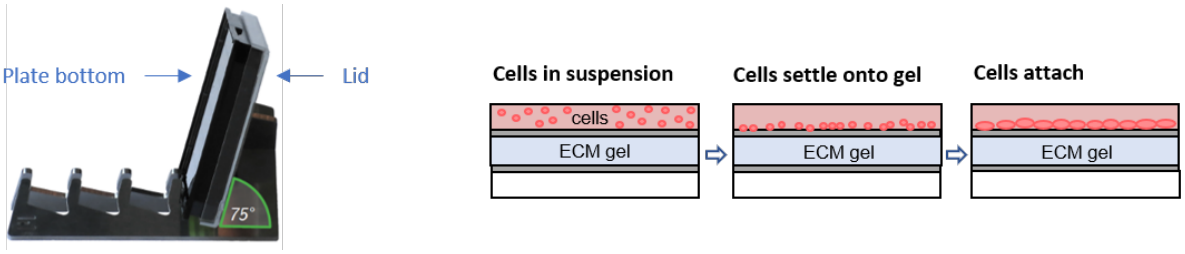

Figure 2: Incubate OrganoPlate® on the side to allow cells to attach to the ECM gel

5. References

Van Duinen, V. *et al.* (2019). Standardized and Scalable Assay to Study Perfused 3D Angiogenic Sprouting of iPSC-Derived Endothelial Cells In Vitro. JoVE.

MIMETAS product list

Cat. No.	Product Name
MI-AR-CC-01	OrganoReady® Caco-2
9605-400-B	OrganoPlate® 2-lane
4004-400-B	OrganoPlate® 3-lane 40
6405-400-B	OrganoPlate® 3-lane 64
6401-400-B	OrganoPlate® Graft
MI-OFPR-S	OrganoFlow® S
MI-OFPR-L	OrganoFlow® L
MI-OT-1	OrganoTEER®

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