1. Objective

Seeding of cells in an extracellular matrix (ECM) gel in an OrganoPlate® 3-lane for 3D cell culture.

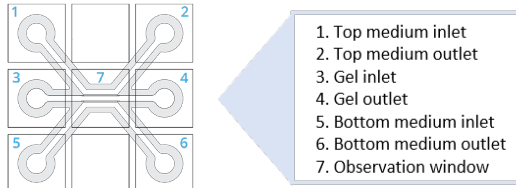


***Figure 1**: Schematic representation of an OrganoPlate® 3-lane tissue chip.*

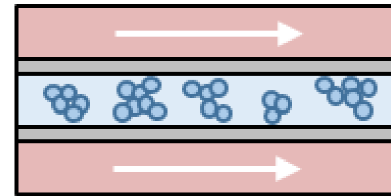


***Figure 2**: Cells are seeded in the gel channel of an OrganoPlate® 3-lane, in an ECM gel, for 3D cell culture. Medium is perfused through the adjacent medium channels.*

2. Background

Cells are grown in an ECM gel to support spheroid formation or other 3D cell interactions/network formations. Morphology can be assessed by phase contrast microscopy and immunostaining followed by fluorescent (confocal) microscopy. 3D culture activity can be measured using different types of read out assays, including fluorescent-label based viability assays, enzymatic luminescent assays, and mRNA expression level analysis.

3. Materials

- OrganoPlate® 3-lane (MIMETAS, 4004-400-B)
- OrganoFlow® S or L (MIMETAS, MI-OFPR-S or MI-OFPR-L)
- The selection of ECM gel and its composition/density is cell type dependent and requires optimization. The components needed for two commonly used ECMs are shown below.
  - Collagen-I 4 mg/mL gel:
    - AMSbio Cultrex® 3D collagen-I rat tail, 5 mg/mL, #3447-020-01
    - 1 M HEPES (ThermoFisher 15630-122, pH 7.2-7.5)
    - 37 g/L NaHCO3 (Sigma S5761-500G, dissolve in sterile MilliQ water, adjust pH to 9.5 using NaOH)
  - Matrigel® (BD, 8-12 mg/ml, #356237)
- Medium (10 mL per OrganoPlate®)
- Cells: seeding density is dependent on the cell type
- HBSS (Sigma H6648)
- Repeating pipette for gel seeding and cell seeding, we recommend:
  - The Eppendorf® Multipette® M4 with the Eppendorf® Biopur® 0.1 mL tip (VWR #613-2067) for dispensation of 2 µL, <u>or</u>
  - The Sartorius eLINE® electronic pipette (Sartorius, #735021 (previously #730021)) for accurate dispensation of volumes ranging from 0.2 to 10 µL. Use with corresponding Sartorius tips or with Eppendorf® ep Dualfilter tips (Eppendorf, 022491211 / 0030077512)
- Multichannel pipette (1200 µL and 300 µL) and multichannel tips
- Crushed ice

4. Procedure

For the procedure of in gel seeding, the cells will be mixed with ECM gel and directly seeded in the gel inlet of the OrganoPlate®. After polymerization of the gel, medium is added to the medium channels and perfusion can be initiated (depending on cell type).

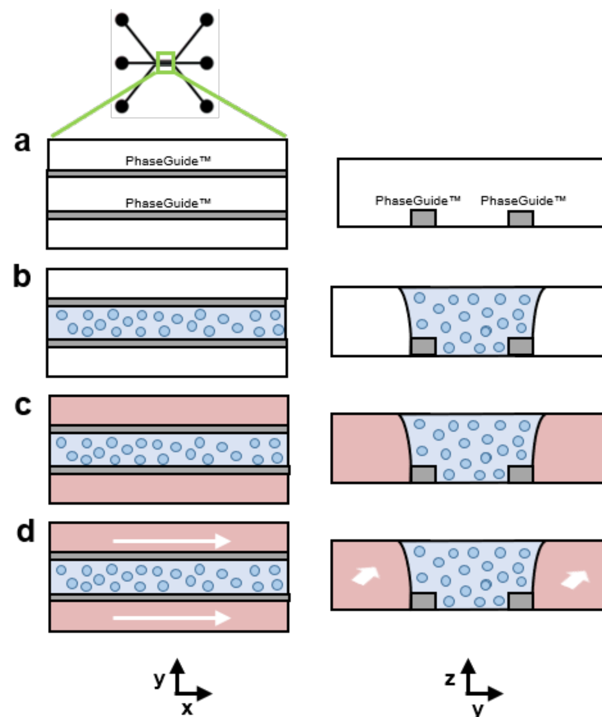


***Figure 3**: Schematic representation of cell seeding in ECM gel in the OrganoPlate® 3-lane*

Harvest and pellet cells

1. Harvest cells according to their dissociation protocol
2. Count the number of live cells in the cell suspension
3. Calculate the required number of cells for seeding in the OrganoPlate® and pellet them
    a. The optimal cell density for seeding in ECM in the OrganoPlate® is cell type dependent (generally between 5,000 and 30,000 cells/µL of gel)
    b. For example:
        i. Number of chips to seed: 40
        ii. Volume to seed per chip: ~2 µL
        iii. Seeding density: 10,000 cells/µL
        iv. You need: 40 x 2 x 10,000 = $8.0*10^5$ cells
        v. Prepare 25% extra: pellet $1.0*10^6$ cells
4. Place tube with pelleted cells on ice.

Seed cells in ECM gel in the OrganoPlate®

Note: avoid touching the bottom glass plate of the OrganoPlate®

1. Take the OrganoPlate® from the packaging
2. Add 50 µL of HBSS to all wells in columns 2, 5, 8, 11, 14, 17, 20, and 23 using a multichannel repeating pipette. These columns contain the chips' observation windows
3. Prepare the required amount of ECM gel (e.g. 2 µL gel per chip + 40% extra)
    a. Collagen-I 4 mg/mL preparation
        i. Place an Eppendorf tube on ice
        ii. The collagen-I 4 mg/mL gel is prepared by mixing 1 M HEPES, 37 g/L $NaHCO_3$, and 5 mg/mL collagen-I in a 1:1:8 ratio. For example, to prepare 100 µL of gel:
            - Place an Eppendorf tube on ice
            - Mix 10 µL of 1 M HEPES with 10 µL of 37 g/L $NaHCO_3$
            - Add 80 µL of collagen-I 5 mg/mL to the HEPES/$NaHCO_3$ mixture
        iii. Prepare at least 100 µL of total gel volume to ensure proper mixing of all components
        iv. Mix well by pipetting the mixture up and down >20 times, while keeping it on ice
        v. If bubbles are formed, quickly spin the tube down (~5 seconds)
        vi. Use gel immediately after preparation (within 10 minutes)
    b. Matrigel® preparation
        i. Thaw Matrigel® on ice overnight
        ii. Depending on the stock concentration, the Matrigel® can be used directly as supplied or it may need to be diluted using **cold** medium
        iii. A Matrigel® concentration of ~9 mg/mL is compatible with most cell types

4. Aspirate medium from the pelleted cells
5. Resuspend pellet in ECM gel to obtain a cell-gel suspension of the desired cell density
    a. For example:
        i. Number of cells in pellet: $1.0*10^6$
        ii. Desired cell density: 10,000 cells/µL gel
        iii. Volume of gel needed: $1.0*10^6/10,000 = 100$ µL gel
        iv. Resuspend the cell pellet in the prepared ECM gel and mix well by pipetting
        v. Keep the cell-gel suspension on ice

6. Dispense the cell-gel suspension into the gel inlet (columns 1, 4, 7, 10, 13, 16, 19, 22; rows B, E, H, K, N) using the Eppendorf® Multipette® M4 or the Sartorius eLINE electronic pipette.
    Gently place your pipette tip on top of the hole in the bottom of the well and dispense the cell-gel suspension. Contact between the pipette tip and the hole is essential for correct seeding. Correct positioning of the cell-gel suspension on top of hole allows capillary forces to pull the suspension into the microfluidic gel channel (see figure 4).
    a. The optimal seeding volume depends on several factors, such as the viscosity of the gel and the temperature in the lab
    b. Start by seeding 2 µL of cell-gel suspension per gel inlet

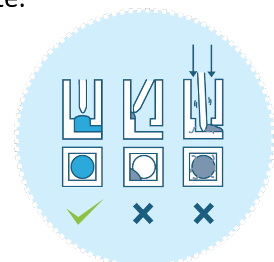


***Figure 4**: Gel loading*

c. In case of incomplete gel filling, increase the seeding volume (i.e. to 2.3 µL)
d. In case the gel overflows from the gel channel into the adjacent medium channel, reduce the seeding volume (i.e. to 1.7 µL)
e. For examples of correct gel filling in the OrganoPlate® 3-lane, see figure 5.
f. Regularly resuspend the cell-gel suspension during seeding to ensure homogenous cell density

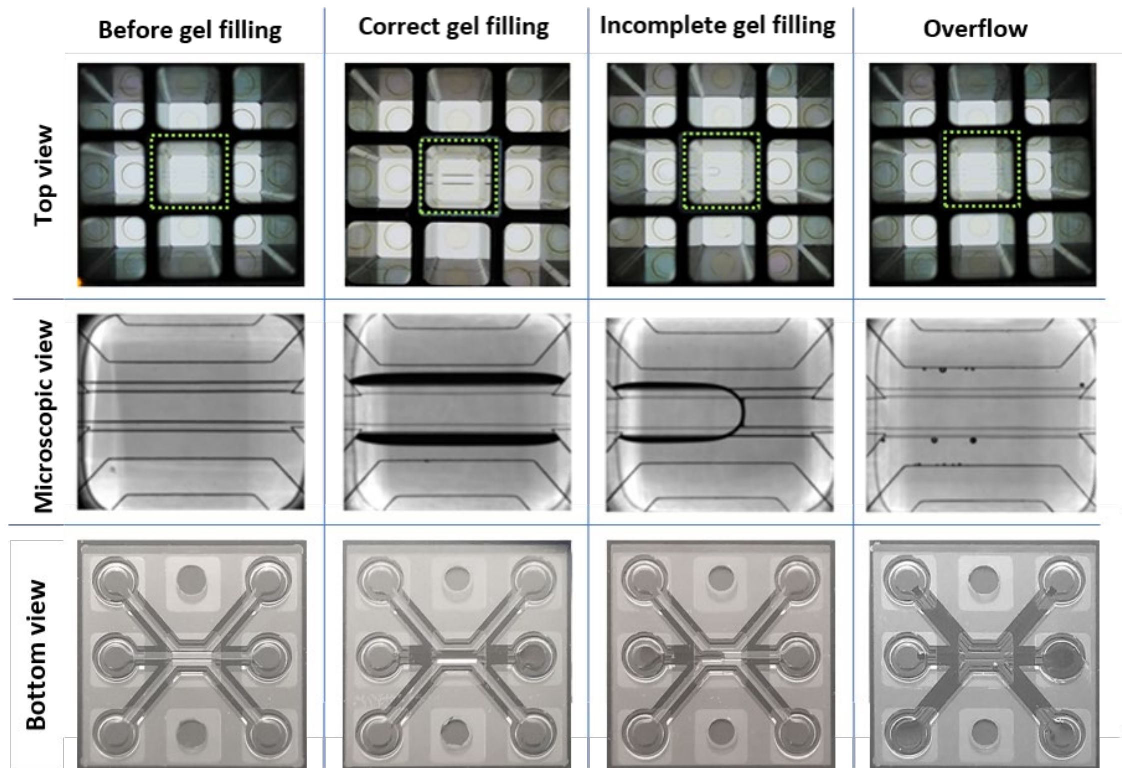


***Figure 5**: Overview of correct gel filling, incomplete gel filling, and overflow in the OrganoPlate® 3-lane.*

Both the Eppendorf® Multipette® M4 and the Sartorius eLINE electronic pipette can successfully be used to seed the OrganoPlate®. Tabel 1 shows an overview of each pipette's advantages and disadvantages for gel seeding.

Table 1

Pipette for gel seeding	Advantage	Disadvantage
Eppendorf® Multipette® M4	Allows user to seed many chips in one go without having to reload the pipette tip*	Only allows whole-microliter volumes (1 µl, 2 µL, etc.), making it more difficult to correct incomplete gel filling or overflow
Sartorius eLINE® electronic pipette	Allows user to select the seeding volume with 10 nL steps, such as 1.75 µL, 1.80 µL, 1.85 µL, etc.	Total volume of pipette is 10 µL, allowing user to seed approximately 5 chips at a time before having to reload the pipette

*We recommend seeding a maximum of 20 chips at once before emptying and reloading the pipette tip with cold gel. This will avoid gelation of the gel while it is in the pipette tip.

7. Place the OrganoPlate® in a humidified incubator (i.e. 37°C, 5% CO_2) for 15 minutes to allow polymerization of the ECM gel
8. Add 50 µL of medium to the top medium inlet (columns 1, 4, 7, 10, 13, 16, 19, 22; rows A, D, G, J, M) and bottom medium inlet (columns 1, 4, 7, 10, 13, 16, 19, 22; rows C, F, I, L, O) and check if the medium channel is filled properly (this can be observed by checking the bottom of the plate; a correctly filled channel appears as dark)
9. Then add 50 µL of medium to the top medium outlet (columns 3, 6, 9, 12, 15, 18, 21, 24; rows A, D, G, J, M) and bottom medium outlet (columns 3, 6, 9, 12, 15, 18, 21, 24; rows C, F, I, L, O)
10. Ensure no air bubbles are trapped in medium in- and outlets. If bubbles are trapped, remove the bubbles gently with a pipette tip.
11. Optional: Place the plate on the OrganoFlow® rocker in a humidified incubator to start cell culture (see figure 6)
    a. An inclination of 7° and an interval of 8 minutes is optimal for many cultures; however, this is cell type dependent.

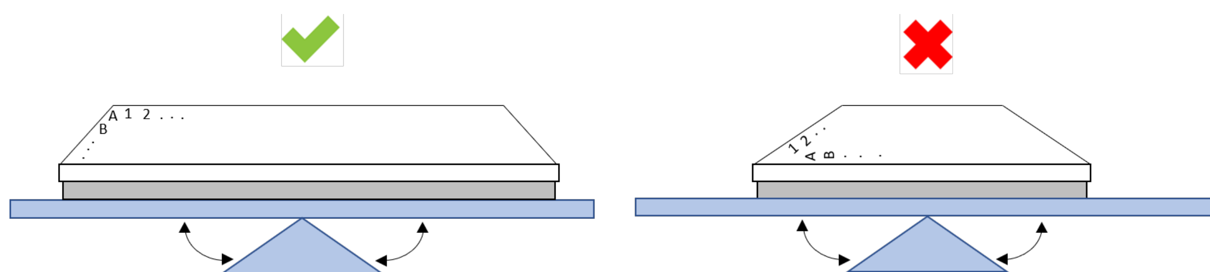


***Figure 6**: Place the OrganoPlate® on the OrganoFlow® in the correct orientation*

12. Refresh medium every 2-3 days by aspirating and replacing the medium from medium inlets and outlets (50 µL in each) using a repeating multichannel pipette.
13. An example of a cells-in-gel culture in the OrganoPlate® 3-lane is shown in figure 7.

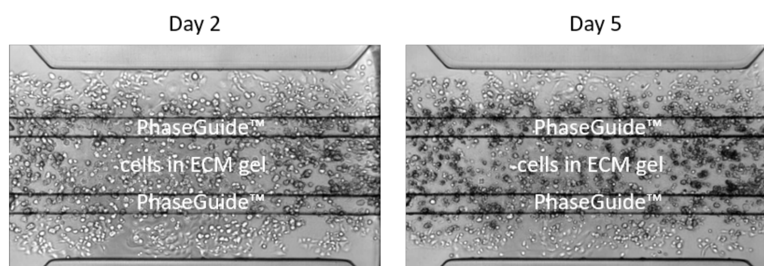
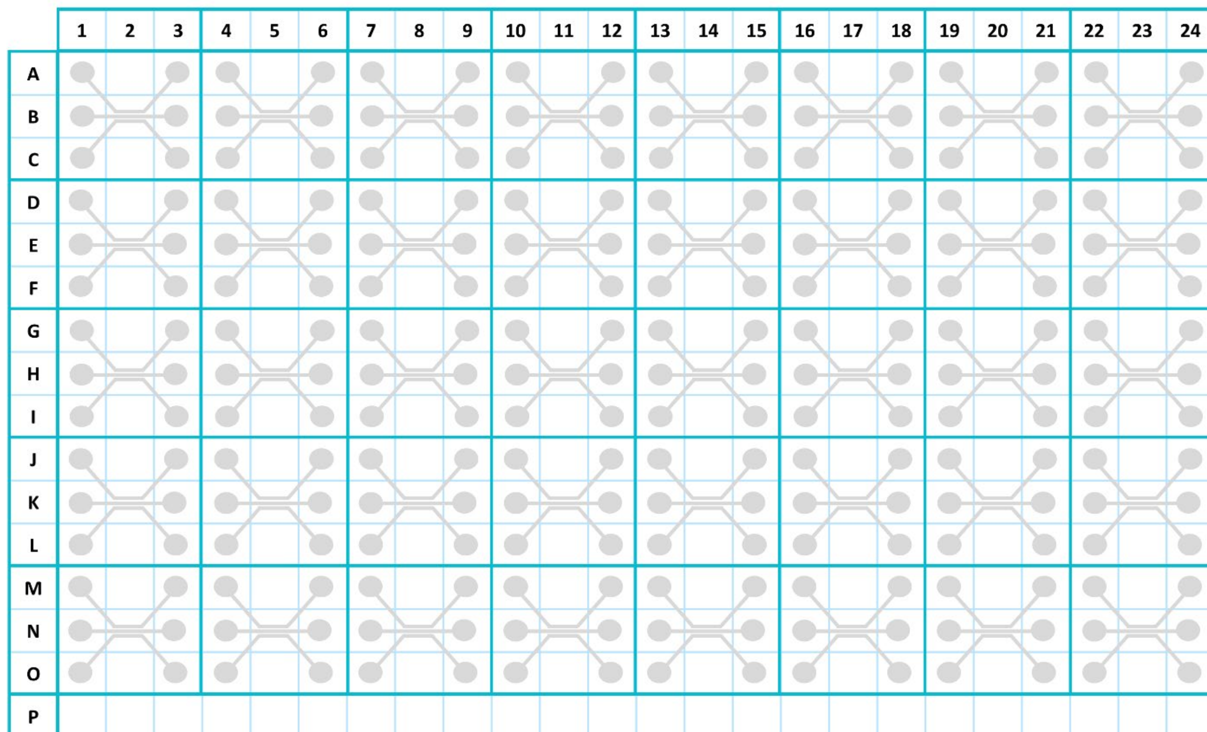


***Figure 7**: Pancreatic cancer cells in Matrigel® in the OrganoPlate® 3-lane*

Plate layout



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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