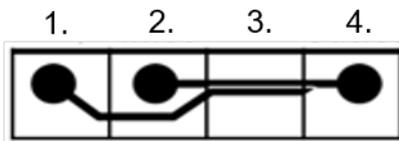


OrganoPlate® 2-lane in-gel seeding

1. Objective

Seeding of cells in an extracellular matrix (ECM) gel in an OrganoPlate® 2-lane for 3D cell culture.



1. Gel inlet
2. Medium inlet
3. Observation window
4. Medium outlet

Figure 1: Schematic representation of an OrganoPlate® 2-lane tissue chip.

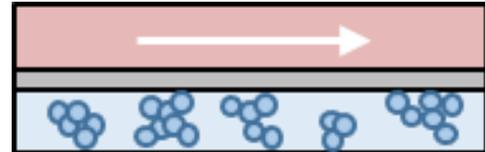


Figure 2: Cells are seeded in the gel channel of a OrganoPlate® 2-lane, in an ECM gel, for 3D cell culture. Medium is perfused through the adjacent medium channel.

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® 2-lane (MIMETAS, 9605-400-B)
- 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 (Life Technologies 15630-122, pH 7.2-7.5)
 - 37 g/L NaHCO₃ (Sigma S5761-500G, dissolve in MilliQ, adjust pH to 9.5 using NaOH)
 - Matrigel® (BD, 8-12 mg/ml, #356237)
- Medium (15 mL per OrganoPlate®)
- Cells: seeding density is dependent on the cell type
- HBSS (Sigma H6648)
- Repeating pipette for gel loading 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, or
 - The Sartorius eLINE® electronic pipette (Sartorius, #735021) 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) with 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 can be applied to the medium channel and perfusion can be initiated (depending on cell type).

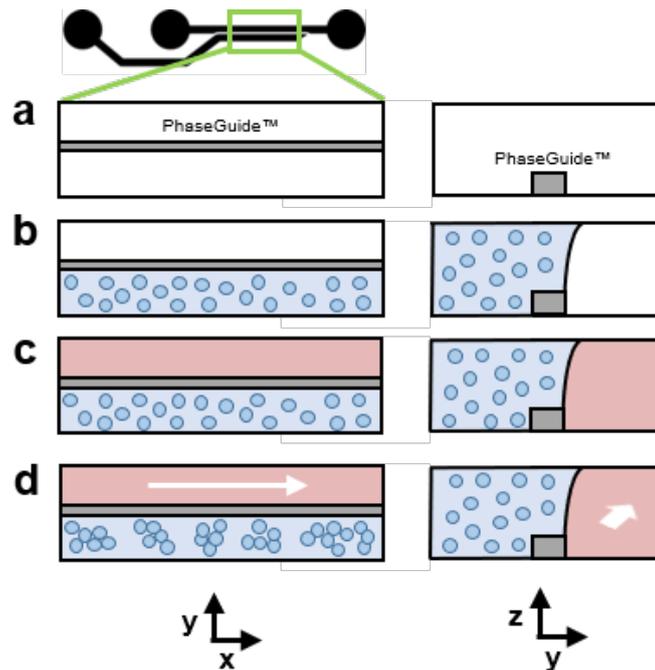


Figure 3: Schematic representation of cell seeding in ECM gel in the OrganoPlate® 2-lane

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: 96
 - ii. Volume to seed per chip: $\sim 2 \mu\text{L}$
 - iii. Seeding density: 10,000 cells/ μL
 - iv. You need: $96 \times 2 \times 10,000 = 1.92 \times 10^6$ cells
 - v. Prepare 25% extra: pellet 2.4×10^6 cells
4. Place tube with pelleted cells on ice.

OrganoPlate® 2-lane in-gel seeding

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 observation windows (columns 3, 7, 11, 15, 19, 23) using a multichannel repeating pipette
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 and place tube back on ice
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: 2.4×10^6
 - ii. Desired cell density: 10,000 cells/ μL gel
 - iii. Volume of gel needed: $2.4 \times 10^6 / 10,000 = 240 \mu\text{L}$ 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, 5, 9, 13, 17, 21) using the Sartorius eLINE electronic pipette.
 - a. 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).
 - b. The optimal seeding volume depends on several factors, such as the viscosity of the gel and the temperature in the lab
 - c. Start by seeding 2 μL of cell-gel suspension per gel inlet
 - d. In case of incomplete gel filling, increase the seeding volume (i.e. to 2.3 μL)

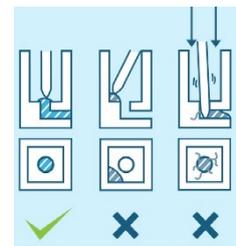


Figure 4: Gel seeding

OrganoPlate® 2-lane in-gel seeding

- e. In case the gel overflows from the gel channel into the adjacent medium channel, reduce the seeding volume (i.e. to 1.7 µL)
- f. For examples of correct gel filling in the 2-lane OrganoPlate®, see figure 5
Note: these images show seeding of a gel without cells and serve only as an example.
- g. Regularly resuspend the cell-gel suspension during seeding to ensure homogenous cell density

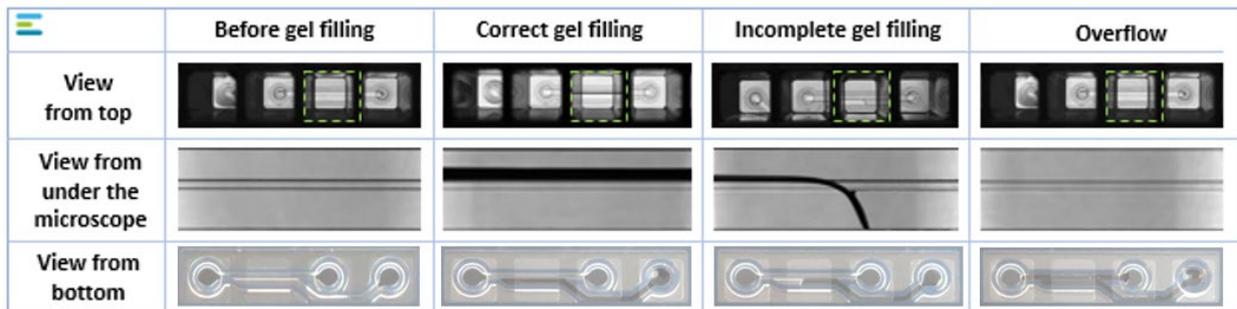


Figure 5: Overview of correct gel filling, incomplete gel filling, and overflow in the OrganoPlate® 2-lane. Note: images show filling of gel without cells and function only as an example to demonstrate correct gel seeding.

Both the Eppendorf® Multipipette® M4 and the Sartorius eLINE electronic pipette can successfully be used to load gel in the OrganoPlate®. Table 1 shows an overview of each pipette’s advantages and disadvantages for gel loading.

Table 1

Pipette for gel loading	Advantage	Disadvantage
Eppendorf® Multipipette® M4	Allows user to load 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 loading 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 load approximately 5 chips at a time before having to reload the pipette

*We recommend loading a maximum of 16 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% CO₂) for 15 minutes to allow polymerization of the ECM gel
8. Add 50 µL of medium to the medium inlet (columns 2, 6, 10, 14, 18, 22) 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 medium outlet (columns 4, 8, 12, 16, 20, 24)
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. Place the plate on the OrganoFlow® 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 most cultures
 - b. Neuronal cultures show optimal results when cultured static (no rocker needed)

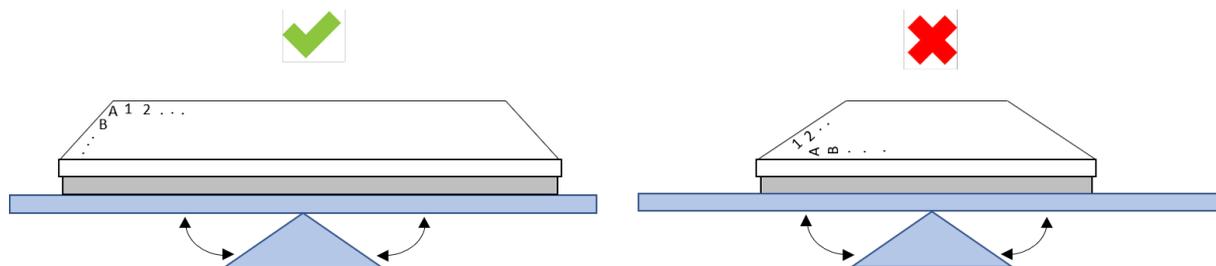


Figure 6: Place the OrganoPlate® on the OrganoFlow® in the correct orientation

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® 2-lane is shown in figure 7.

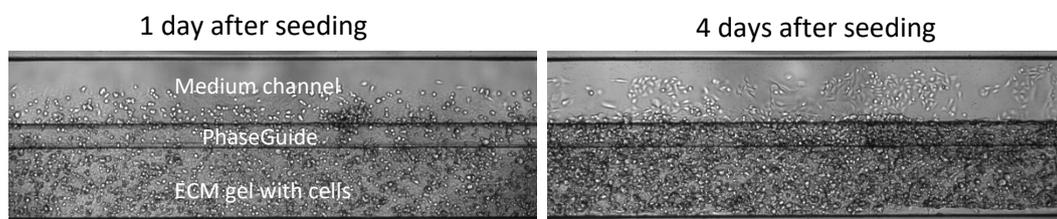


Figure 7: Pancreatic cancer cells cultured in collagen-I in the OrganoPlate® 2-lane

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

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®

Contact information

For questions, please contact us through the e-mail addresses stated below

Purchasing: order@mimetas.com

Customer service: info@mimetas.com

Technical support: support@mimetas.com

MIMETAS Europe

J.H. Oortweg 19
2333 CH, Leiden
The Netherlands
Phone: +31 (0)85 888 3161

MIMETAS USA

704 Quince Orchard Road
Suite 260, MD 20878
Gaithersburg, USA
+1 (833) 646-3827

MIMETAS Japan

4F Tekko Building,
1-8-2 Marunouchi, Chiyoda-Ku
Tokyo, 100-0005, Japan
+81 3-6870-7235

This protocol is provided 'as is' and without any warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose or assured results, or that the use of the protocol will not infringe any patent, copyright, trademark, or other proprietary rights. This protocol cannot be used for diagnostic purposes or be resold. The use of this protocol is subject to Mimetas' General Terms and Conditions of Delivery, Purchase and Use.

MIMETAS®, OrganoPlate®, OrganoFlow®, OrganoReady®, and OrganoTEER® are registered trademarks of MIMETAS BV. Cultrex® is a registered trademark of Trevigen, Inc. Matrigel® is a registered trademark of Corning, Inc. eLINE® is a registered trademark of Sartorius Biohit Liquid Handling Oy. Eppendorf® is a registered trademark of Eppendorf AG, Germany.