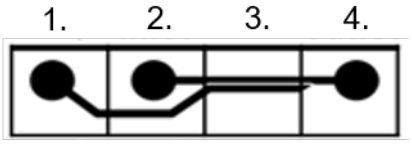
1. Objective

Seeding of cells of the central nervous system (CNS) in an extracellular matrix (ECM) gel in OrganoPlate® 2-lane for 3D cell culture (as published by Wevers et al., DOI: 10.1038/srep38856).



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

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

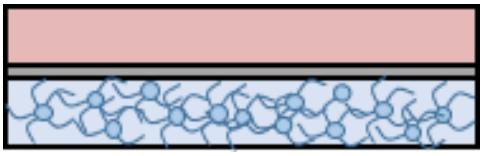


***Figure 2**: CNS cells are seeded in the gel channel of an OrganoPlate® 2-lane, in an ECM gel, for 3D cell culture. Medium is added to the adjacent medium channel.*

2. Background

Cells of the CNS, such as neurons, astrocytes, or neural stem cells, are grown in an ECM gel to support formation of 3D networks. 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, 9603-500-B)
- Matrigel®-GFR (Corning, 356231)
- Medium (15 mL per OrganoPlate®)
- CNS cells
- HBSS (Sigma H6648)
- Repeating pipette for gel seeding, we recommend the Sartorius eLINE® electronic pipette (Sartorius, #735021 (previously #730021)) for accurate dispensation of volumes 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)
- Multichannel tips
- Crushed ice
- OrganoFlow® (for neuron-astrocyte co-culture; MIMETAS, MI-OPFR)

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.

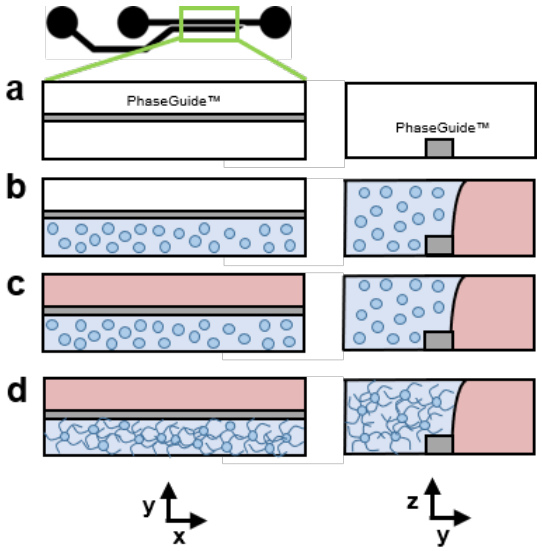


***Figure 3**: Schematic representation of CNS cell seeding in ECM gel in the OrganoPlate® 2-lane*

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 cell density for CNS cell seeding in the OrganoPlate® may require optimization for specific cell types. We recommend starting with a density of 25,000 cells/µL gel. For neuron-astrocyte co-culture, a mixture of 85% neurons and 15% astrocyte ratio was optimized.
    b. For example:
        i. Number of chips to seed: 96
        ii. Volume to seed per chip: ~2 µL
        iii. Seeding density: 25,000 cells/µL
        iv. You need: 96 x 2 x 25,000 = $4.8*10^6$ cells
        v. Prepare 25% extra: pellet $6*10^6$ 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 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. Matrigel®-GFR preparation
        i. Thaw Matrigel®-GFR on ice overnight
        ii. Depending on the stock concentration, the Matrigel®-GFR can be used directly as supplied or it may need to be diluted using **cold** medium
        iii. A Matrigel®-GFR concentration of 7-9 mg/mL is compatible with most CNS 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: $6*10^6$
        ii. Desired cell density: 25,000 cells/µL gel
        iii. Volume of gel needed: $6*10^6/25{,}000$ = 240 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, 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)
    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

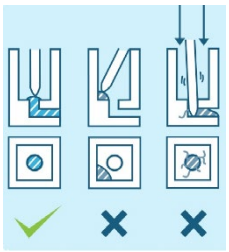


***Figure 4**: Gel seeding*

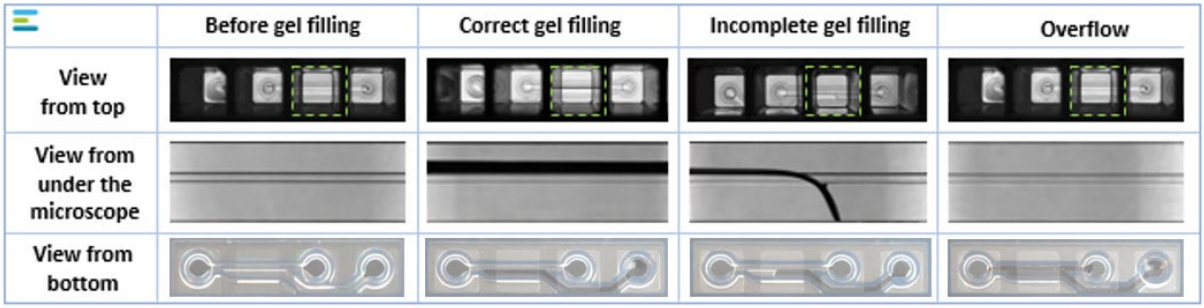


***Figure 5**: Overview of correct gel filling, incomplete gel filling, and overflow in the OrganoPlate® 2-lane. Note: images show filling of gel without cells and function only as an example to demonstrate correct gel seeding.*

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 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)
    a. Optional: also add 50 µL to the gel inlet (columns 1, 5, 9, 13, 17, 21)
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 plate in incubator to start culture
    a. For neuron-astrocyte co-culture, place the OrganoPlate® on the OrganoFlow® (2°, 60 min)
    b. For neuron monoculture, culture static
12. Refresh medium every 2-3 days by aspirating and replacing the medium from medium inlets and outlets and optionally gel inlets (50 µL in each) using a repeating multichannel pipette
13. An example of a CNS cells-in-gel culture in the OrganoPlate® 2-lane is shown in figure 6.

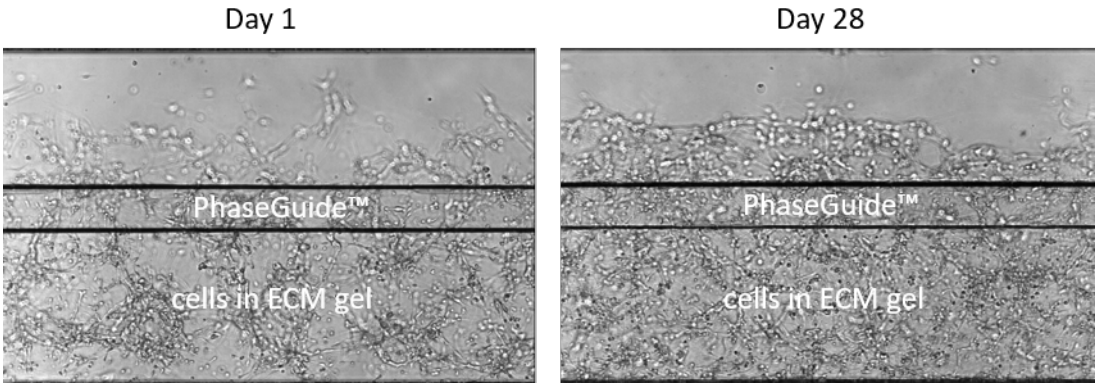
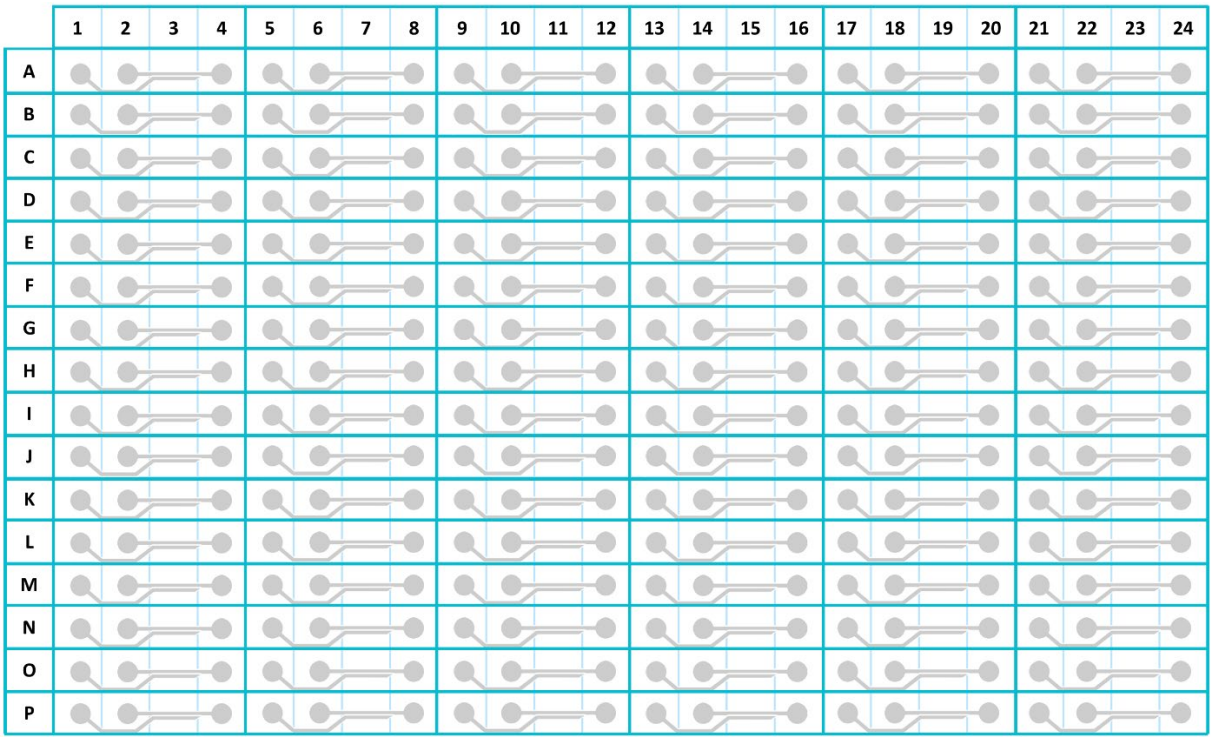


***Figure 6**: Culture of neural stem cells in Matrigel® in the OrganoPlate®2-lane*

Plate layout



MMIMETAS 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