1. Objective

This assay is performed to assess the barrier function and lumen formation of endothelial vessels grown in the OrganoPlate® Graft and can be used:

- As a criterium for optimizing a vascular model
- To detect compound-induced effects on the vessels (end-point or in real-time)

2. Background

In the OrganoPlate® Graft, endothelial cells are seeded against an ECM gel to establish an endothelial barrier tissue. Addition of pro-angiogenic factors triggers the formation of angiogenic sprouts, resulting in a vascular network. The barrier function and the presence of perfusable lumina can be assessed by perfusing the endothelial vessels with a fluorescent dye and monitoring the distribution of the dye through the chip.

The fluorescent dye can be added to:

- Both perfusion channels to assess barrier function of the vessels and presence of perfusable lumina in the vascular network (see figure 1a)
- One of the two perfusion channels to assess vessel connectivity in the middle of the Graft gel chamber (see figure 1b)

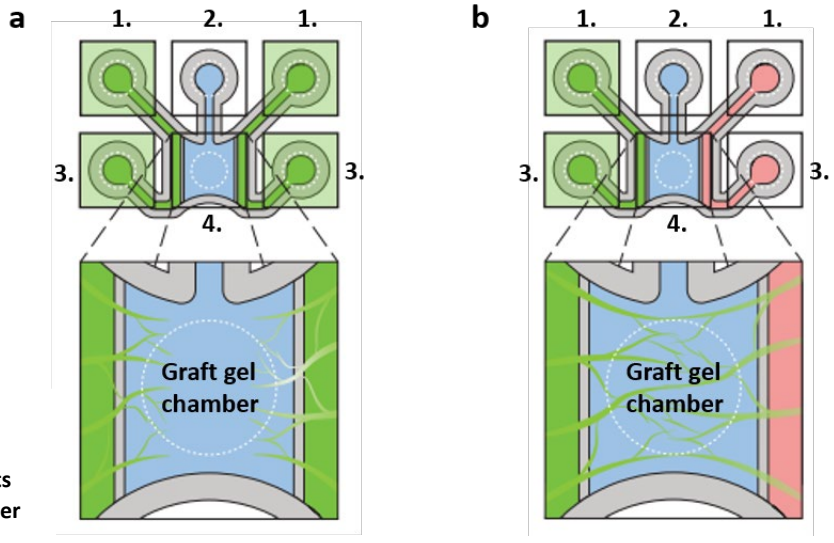


***Figure 1**: Schematic overview of the OrganoPlate® Graft perfusion assay. **a)** Vascular network in the OrganoPlate® Graft in which the angiogenic sprouts from both sides have not connected in the middle of the Graft gel chamber. Fluorescent dye is added to both perfusion channels to evaluate barrier function and perfusability of the vessels. **b)** Vascular network in the OrganoPlate® Graft in which the angiogenic sprouts from both sides have connected in the middle of the Graft gel chamber. Fluorescent dye is added to one of the two perfusion channels to evaluate barrier function, perfusability, and vessel connectivity.*

3. Materials

- OrganoPlate® Graft (MIMETAS, 6401-400-B) with a vascular network
- Medium
- Fluorescent compound
    a. i.e. FITC-dextran 150 kDa (Sigma, 46946, stock solution 25 mg/mL in HBSS)
    b. Keep all solutions sterile

4. Assay

1. Set the microscope to allow image acquisition to start as soon as dyes are added to the chips

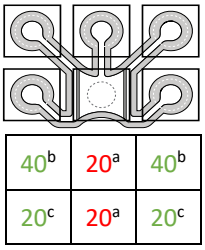
2. Prepare a fluorescent working solution containing the fluorescent compound
    a. The final concentration of the fluorescent compounds is 0.5 mg/mL in medium, requiring a 50x dilution from stock
    b. One OrganoPlate® Graft requires
        i. 120 µL working solution and 40 µL medium per chip, when dye is added to both perfusion channels
        ii. 60 µL working solution and 80 µL medium per chip, when dye is added to one of the two perfusion channels

3. Aspirate medium from all inlets and outlets

4. Start assay by pipetting the following solutions in this specific order:

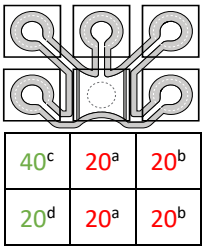
Option 1: dye in both perfusion channels

a. Pipet 20 µL medium in the Graft gel chamber and gel inlet
b. Pipet 40 µL of the working solution in the perfusion inlets of both perfusion channels
c. Pipet 20 µL of the working solution working solution in the perfusion outlets of both perfusion channels
d. Proceed to image acquisition



Option 2: dye in one of the two perfusion channels (left in this example)

a. Pipet 20 µL medium in the Graft gel chamber and gel inlet
b. Pipet 20 µL medium in the perfusion inlet and the perfusion outlet of the right perfusion channel
c. Pipet 40 µL of the working solution in the perfusion inlet of the left perfusion channel
d. Pipet 20 µL of the working solution in the perfusion outlet of the left perfusion channel
e. Proceed to image acquisition



5. Image acquisition

This assay can be imaged using a (high-content) fluorescent microscope. The exact protocol for your specific imaging system may require optimization. Keep the following points in mind when imaging the perfusion assay:

1. Make sure the dye is in focus when imaging. Image-based focusing algorithms can struggle with large fronts of dye. An alternative here is to turn off image-based auto-focusing and pre-define a single focal point (also known as Z-location). Laser-based focusing systems usually have no issues with this assay as they are unaffected by the presence of dye inside the channel.

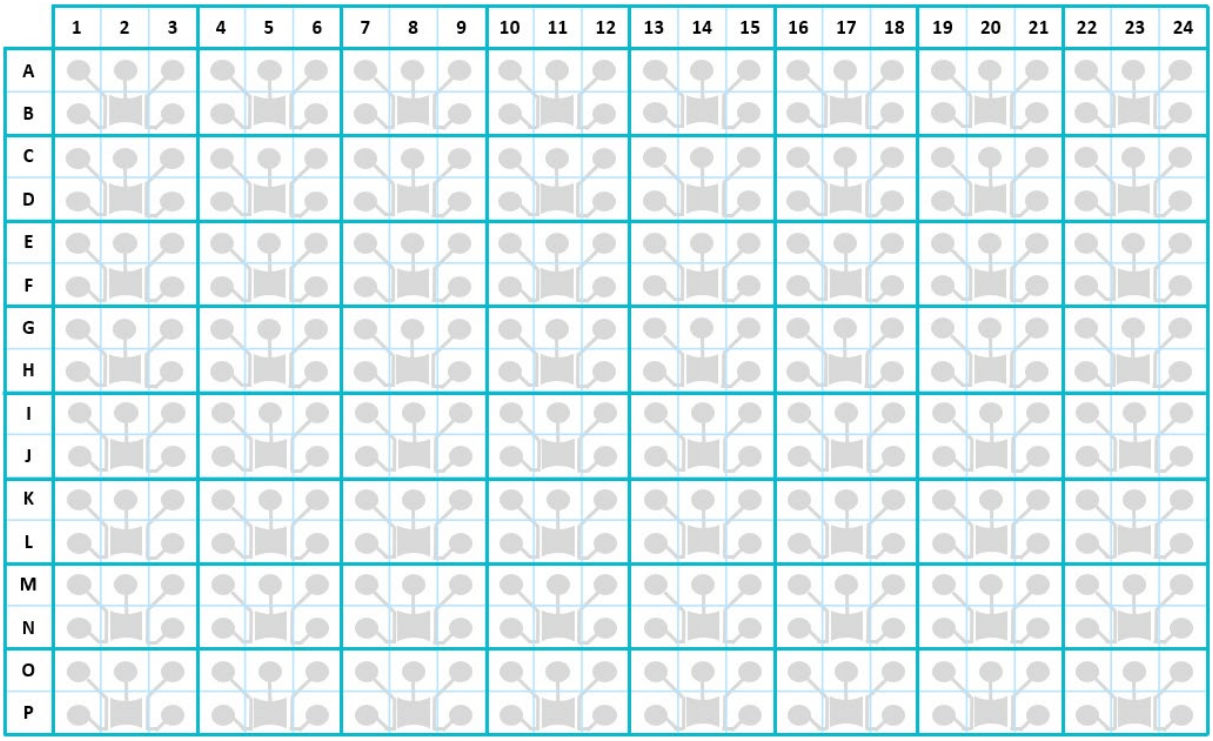
2. Make sure not to over-expose the fluorescent dyes when setting the exposure times. Saturated signals will hamper correct quantification of the assay in a later stage.

3. You can choose to do an end-point measurement only, or to monitor perfusion over time. To do the latter, image each chip at several set time points. Depending on the speed of the microscope, you can for example image each chip every 15-30 sec for the total duration of the assay (*i.e*. 30 minutes).

6. Optional: continue culture

To continue culture of the tubes after performing the perfusion assay, aspirate all solutions from the wells and add replace with medium (with or without pro-angiogenic factors, depending on the experimental design). Then place the OrganoPlate® Graft back on the MIMETAS rocker in the incubator to continue culture.

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®

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