1. Objective

This protocol describes the procedure of placing and culturing tissue fragments (e.g. PDX (patient derived xenograft) materials, spheroids, or organoids) in the OrganoPlate® Graft in presence of endothelial tubules or a vascular network.

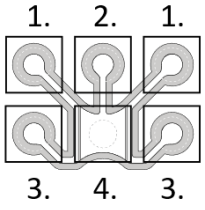
2. Background

The OrganoPlate® Graft (see figure 1) can be used to grow vascular tubules that resemble blood vessels *in vivo* by seeding endothelial cells in the perfusion channels. When the endothelial tubules are formed, pro-angiogenic factors can be added to trigger the formation of angiogenic sprouts, resulting in a vascular network (see figure 2). This protocol describes the procedure of placing tissue fragments in the Graft gel chamber of the OrganoPlate® Graft to study the angiogenic/vasculogenic properties of the tissue.

Tissue placement and culture conditions may differ depending on the properties of the tissue. For example, tissues with a size < 1 mm do not require any cutting and/or ECM embedding prior to placement in the OrganoPlate® Graft, while tissues with a size > 1 mm usually require reduction in size and embedding in ECM. Two separate sections can be found in this protocol:

a) The first section describes the placement procedure for tissues that do not require cutting and ECM embedding.
b) The second section describes the placement procedure for tissues that do require cutting and ECM embedding.

When using different tissues, optimization of culture conditions may be required.



1. Top inlets
2. Gel inlet
3. Bottom outlets
4. Graft chamber with inlet

***Figure 1**: Schematic representation of an OrganoPlate® Graft tissue chip.*

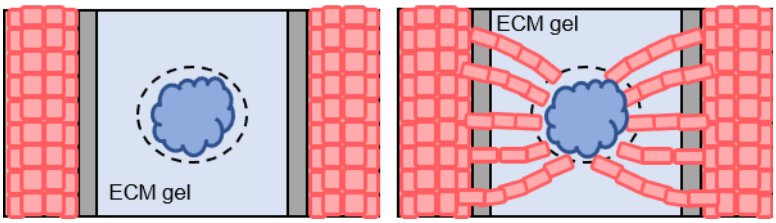


***Figure 2**: An ECM gel is seeded in the Graft chamber. Endothelial cells are added to the perfusion channels and form endothelial tubules (left panel). If desired, a pro-angiogenic cocktail can be applied to induce formation of a vascular network (right panel). Tissues (i.e. PDX fragments, spheroids, or organoids) can be placed in the Graft chamber to study the angiogenic/vasculogenic properties of the tissues.*

3. Materials

- OrganoPlate® Graft (MIMETAS, 6401-400-B) with or without a vascular network
  - Procedure for formation of a vascular network is described in the following protocol: *OrganoPlate® Graft Vascular Network Formation*
- Medium: 14 mL for all medium in- and outlets and 4 mL for all Graft chambers
- Matrigel® Growth Factor Reduced (Matrigel®-GFR, 7-8 mg/mL, Corning #356237)
- 1x PBS
- Multichannel pipette (1200 µL and 300 µL)
- Multichannel tips
- Crushed ice
- Small petri dishes
- Disposable blades
- Tools for tissue positioning, e.g. precision tools or disposable needles (0.3x13 mm, REF 304000, BD biolance™3) and syringes
- P200 pipette and wide bore pipette tips (Pure™ 200G, VWR, #53225-682)
- Endothelial cell medium
  - i.e. MV2 medium (PromoCell, C-22221)
- Tissue specific media
- Angiogenic cocktail if sprouts induction through pro-angiogenic factors is desired
  - Contents are described in protocol *OrganoPlate® Graft Vascular Network Formation*

4. Tissue placement

a) Tissues that do not require cutting (tissues < 1mm) and ECM embedding

1. Aspirate the media from all Graft chambers and all perfusion inlet and outlet wells
2. Add 50 µL of endothelial cell specific medium in all perfusion inlet and outlet wells
3. Add 50 µL of tissue specific media in all Graft chambers
4. Transfer tissues to Graft chambers (rows B, D, F, H, J, K, L, N, P; columns 2, 5, 8, 11, 14, 17, 20, 23, **see plate layout on pg.5**) using wide bore pipette tips:
   a. E.g. tissues grown in a wells plate
   b. Set a p200 pipette to 50 µL and place the pipette tip in proximity of the tissue
   c. Pipette up 50 µL of media and ensure the tissue is taken up in the pipette tip during this procedure
   d. Let the tissue sink to the bottom of the pipette tip
   e. Position the pipette tip on top of the Graft chamber and gently touch the surface of the media present in the Graft chamber with the pipette tip
   f. Dispense the tissue and allow it to fall in the middle of the Graft chamber (on top of the hole)

- Try to only dispense the tissue from the pipette tip and not the remaining 50 µL of media present in the tip, as dispensation of this medium into the medium already present in the well can cause the freshly placed tissue to be displaced

g. Repeat steps a-f for all chips in the OrganoPlate® Graft

5. Place the plate back in the incubator on the MIMETAS rocker (14° inclination, 8 min interval)
6. Observe cultures daily and take pictures. Refresh medium every 2-3 days
    a. Medium is refreshed by aspirating media from all inlet and outlet wells and the Graft chamber and replacing it with 50 µL of fresh media. Generally, tissues attach well and are not disturbed by this procedure when performed gently

b) Tissues that do require cutting (tissues > 1 mm) and ECM embedding

1. Reduce the size of the tissue using a blade or needles
2. Cover the tissue fragments with tissue specific medium or PBS to prevent them from drying out
3. In the OrganoPlate®, aspirate media from all Graft chambers and all perfusion inlet and outlet wells
4. Transfer the tissue fragments to the Graft chambers (rows B, D, F, H, J, K, L, N, P; columns 2, 5, 8, 11, 14, 17, 20, 23) on top of the collagen-I gel (see figure 3)
    a. Pick up the tissue fragment by gently touching it with a pipette tip (i.e. p2.5 pipette and a thin tip), a precision tool or a needle. Transfer the tissue fragment to the OrganoPlate® Graft by gently letting it touch the gel inside the Graft chamber
    b. In case of incorrect positioning, the fragment can be moved to the correct position using the tools used for placement of the fragment

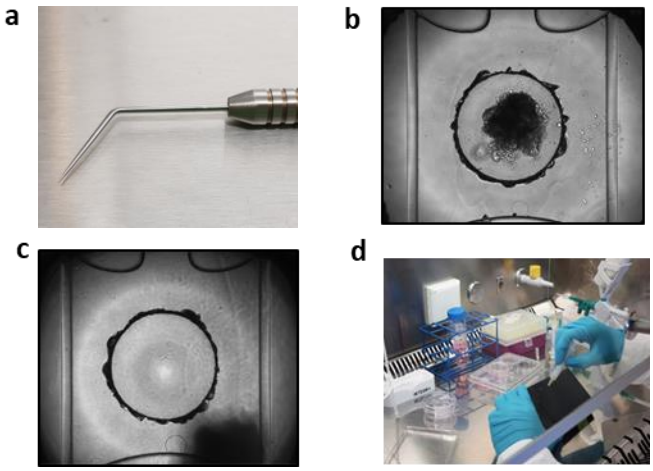


Figure 3: *(**a**) Tissue fragments are transferred to the Graft chamber using a thin precision tool, pipette tip or a bend needle. (**b,c**) Representative pictures of a correctly (b) and incorrectly (c) placed tissue fragment in the Graft chamber (in absence of endothelial cells). (**d**) In case of incorrect positioning, the fragment can be moved to the correct position by using a syringe and a needle.*

5. Add 10 µL of Matrigel®-GFR to all Graft chambers to embed the freshly placed tissues
    a. Keep the Matrigel®-GFR aliquot on ice during the procedure to prevent polymerization
    b. Depending on the tissue, the type of ECM and volume of ECM used for embedding may require optimization
6. Incubate the OrganoPlate® Graft for 30 minutes at 37°C in a humidified incubator to allow polymerization of the Matrigel®-GFR
7. Add 50 µL of tissue specific medium or endothelial medium with or without pro-angiogenic factors to all Graft chambers
    a. Medium optimization is required in case tissue fragments are not compatible with endothelial medium
8. Place the OrganoPlate® Graft back in the incubator on the rocker (14° inclination, 8 min interval)
9. Observe cultures daily and take pictures. Refresh the medium every 2-3 days
    a. Medium is refreshed by aspirating media from all inlet and outlet wells and the Graft chamber and replacing it with 50 µL of fresh media

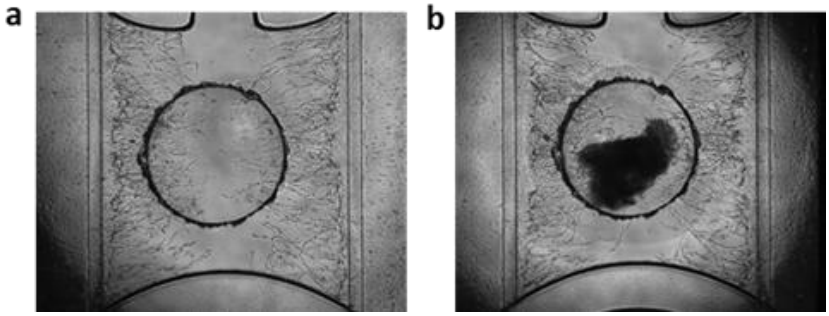


***Figure 4: a**) A vascular network in the OrganoPlate® Graft. **b**) A vascular network in the OrganoPlate® Graft with a tissue fragment placed on top.*

5. Troubleshooting

Tissue displacement: non-ECM embedded

When a tissue is placed in the graft chamber that contains medium (section Tissue placement – tissues that do not require […] embedding), the tissue should be well centred in the chamber (figure 5a). When the tissue is incorrectly positioned (figure 5b), the position can be corrected using an inverted microscope (placed in a sterile environment) and a set of dental tools (figures 6 and 3a).

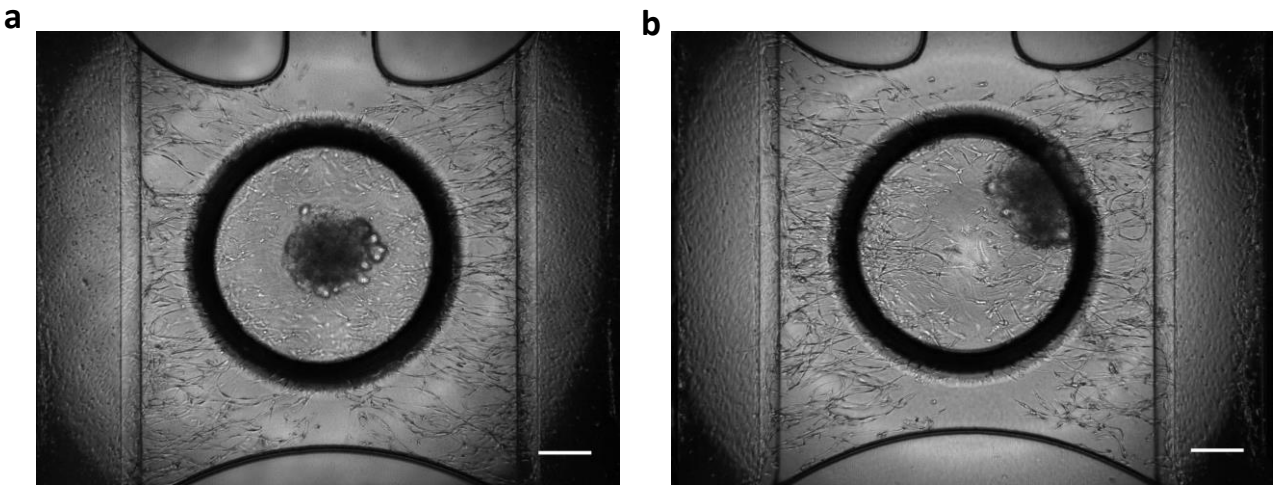


Figure 5: a) a tissue placed correctly in the middle of the graft chamber. b) a displaced tissue that requires correction of position.

Figure 6. An example of an inverted microscope (EVOS XL core) that can support the correction of displaced tissue.

Tissue displacement: ECM embedded

Sometimes the tissue can displace when it is embedded in ECM and after polymerization, medium is added to the graft chamber (see Section *Tissue placement – tissues that require […] embedding, step 7*). Usually, this means the ECM was not solidified well. Potential causes for this include:

1. Presence of liquid in the perfusion channels and graft chamber: if medium is present in the perfusion channels and/or graft chamber (as well as in the inlets and outlets), this may dilute the ECM gel used for embedding. To prevent residual media from interfering with the solidification of the ECM gel, aspirate all the inlet- and outlet wells (from perfusion channels and graft chambers) properly before adding the tissue and ECM gel.

2. Insufficient polymerization time: different types of ECM gel can be used for tissue embedding. Each ECM gel requires a specific amount of time to solidify. Use the polymerization time indicated on the product sheet from the supplier to ensure proper polymerization of the ECM prior to addition of the medium to the graft chamber to avoid tissue displacement.

Loss of tissue (3D) structure

In some cases, the tissue can show 2D outgrowth or lose its 3D structure after placement in the graft chamber. The extend of 2D growth can be tissue-dependent, with some cell types migrating out of the tissue and growing on the collagen gel and/or forming a monolayer on top of the glass.

When the loss of 3D structure is significant and affects the characteristics of the tissue, embedding in an ECM gel can help to prevent this (see section Tissue placement – tissues that require [...] embedding). Depending on the tissue type and longevity of the co-culture, two situations that may require ECM embedding:

1. Non-embedded tissue/short-term culture: The tissue was not embedded in ECM when placed in the graft chamber, and after grafting the tissue showed extensive 2D outgrowth and/or loss of 3D structure. The advice would be to perform ECM embedding from the beginning of the co-culture.

2. Embedded tissue/long-term culture: The tissue was embedded in ECM when placed in the graft chamber, but during culture, extensive 2D outgrowth is observed or 3D structure is lost. It is possible that during long-term culture (>14 days), the ECM gel that was used to embed the tissue got washed away during the medium changes. It is advised to add extra ECM gel after 10 days of culture (after placement of tissue) or prior to the moment that the loss of 3D structure is usually observed. The procedure for this is similar to that of regular ECM embedding.

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																								
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www.mimetas.com | info@mimetas.com | +31 85 888 3161

MIMETAS product list

Cat. No.	Product Name
MI-AR-CC-01	OrganoReady® Caco-2
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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