

1. Objective

This protocol describes the formation of angiogenic sprouts from endothelial vessels grown in the OrganoPlate® 3-lane (based on the [publication](#) by Van Duinen et al., DOI: 10.1007/s10456-018-9647-0).

This protocol has been optimized for human umbilical vein endothelial cells (HUVECs, Lonza). Application of this protocol to other endothelial cell sources may require optimization of procedures, timelines, and reagents.

2. Background

The OrganoPlate® allows culture of perfusable endothelial tubules against an extracellular matrix (ECM) gel. To study angiogenic sprouting, the growth of new blood vessels from pre-existing vessels, a gradient of angiogenic factors is employed. This gradient induces the formation of tip cells and perfusable angiogenic sprouts in the OrganoPlate® 3-lane.

3. Materials

- OrganoPlate® 3-lane with a vessel of Lonza HUVECs (C2519AS) in the top channel (see figure 1)
- EGM-2 medium (Lonza, CC-3162)
- Components for angiogenic cocktail (see table 1)

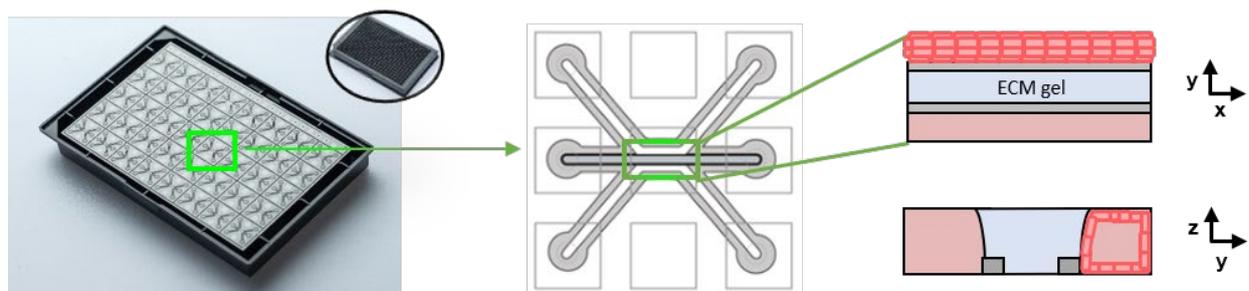


Figure 1: Endothelial tubules in the OrganoPlate® 3-lane. The OrganoPlate® 3-lane holds 40 microfluidic chips that can be used to culture miniaturized tissue- and organ models. Each chip holds three channels. The middle channel is used to seed an extracellular matrix (ECM) gel and the top channel can be used to grow an endothelial vessel. The bottom channel can be used to supply cells with medium or to add compounds, such as an angiogenic cocktail.

4. Procedures

1. Grow endothelial tubules in the top channel of an OrganoPlate® 3-lane
 - a. See protocol *Tubule seeding – 3-lane OrganoPlate®*
 - b. Depending on the cell type, tubule formation can take 2-5 days (~3 days for Lonza HUVEC)

2. Prepare the stock solutions of the six angiogenic compounds as described in column 4 of table 1.
3. Prepare the final angiogenic cocktail (you need 100 µL per chip) by diluting the six angiogenic factors in EGM-2 medium according to the final concentration listed in the last column of table 1.
 - a. Prepare 100 µL per chip + 20% extra volume (~4.5 mL for a complete OrganoPlate® 3-lane)
 - b. If stored at 4°C, the angiogenic cocktail can be used for one week
 - c. When using other cell types than Lonza HUVECs, optimization of medium and angiogenic cocktail composition may be required

Table 1: preparation of angiogenic cocktail

Compound	Supplier	Cat. No.	Stock	Stock storage	Final conc.
rhVEGF-165	Peprtech	100-20	100 µg/mL in 0.1% BSA in PBS	-20°C	37.5 ng/mL
S1P	Sigma-Aldrich	S9666	1 mM in 95% DMSO/5% HCl 1M	-80°C	250 nM
PMA	Sigma-Aldrich	P1585	10 µg/mL 0.1% DMSO in MiliQ	-80°C	37.5 ng/mL
rhFGFb	Peprtech	100-18B	50 µg/mL in 0.1% BSA in PBS	-20°C	37.5 ng/mL
rhMCP-1	ImmunoTools	11343384	100 µg/mL in 0.1% BSA in PBS	-20°C	37.5 ng/mL
rhHGF	ImmunoTools	11343413	100 µg/mL 0.1% BSA in PBS	-20°C	37.5 ng/mL

4. Aspirate medium from all inlets and outlets
5. Add medium without angiogenic factors in the top medium inlets and outlets (50 µL in each)
6. Add the angiogenic cocktail in the bottom medium inlets and outlets (50 µL in each)
 - a. Add first to the inlet, then to the outlet to ensure that no air bubbles are trapped in channel
5. Place the OrganoPlate® back on the MIMETAS rocker platform in the incubator to start perfusion
6. Perform medium changes every 2-3 days (refreshing the regular culture medium in the top channel and the angiogenic cocktail in the bottom channel)
7. Continue exposure to the angiogenic cocktail until endothelial sprouts reach the bottom channel

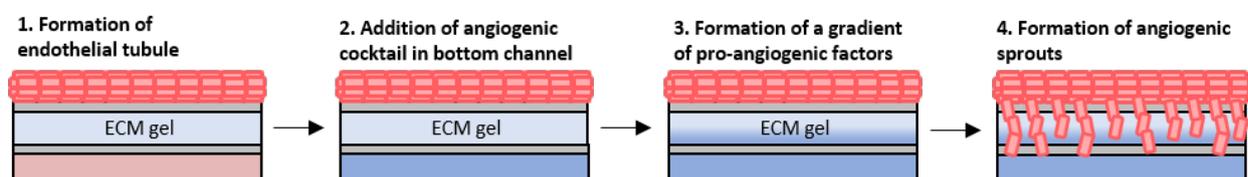


Figure 2: Formation of angiogenic sprouts in the OrganoPlate® 3-lane. Initially, a tubule of endothelial cells is grown in the top channel of the chip against an ECM gel. Next, an angiogenic cocktail is added to the bottom channel and a gradient of pro-angiogenic factors is formed. Endothelial cells respond to the gradient by forming perfusable angiogenic sprouts that extend towards the bottom channel.

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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