1. Objective

This protocol describes the formation of angiogenic sprouts from endothelial vessels grown in the OrganoPlate® 3-lane 64 (based on a publication by Van Duinen et al. using the OrganoPlate® 3-lane 40).

This protocol has been optimized specifically for angiogenic sprouting of primary human umbilical vein endothelial cells (HUVECs). Different applications and endothelial cell sources may require optimization of procedures, timelines, and reagents.

2. Background

The OrganoPlate® allows culture of perfused 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, followed by stalk cells, resulting in perfusable angiogenic sprouts in the OrganoPlate® 3-lane 64.

3. Materials

- OrganoPlate® 3-lane 64 with an endothelial vessel (e.g. Lonza HUVECs cat. C2519AS) (see figure 1)
- Endothelial culture medium (e.g. Lonza EGM-2 cat. CC-3162)
- Components for angiogenic cocktail (see table 1)

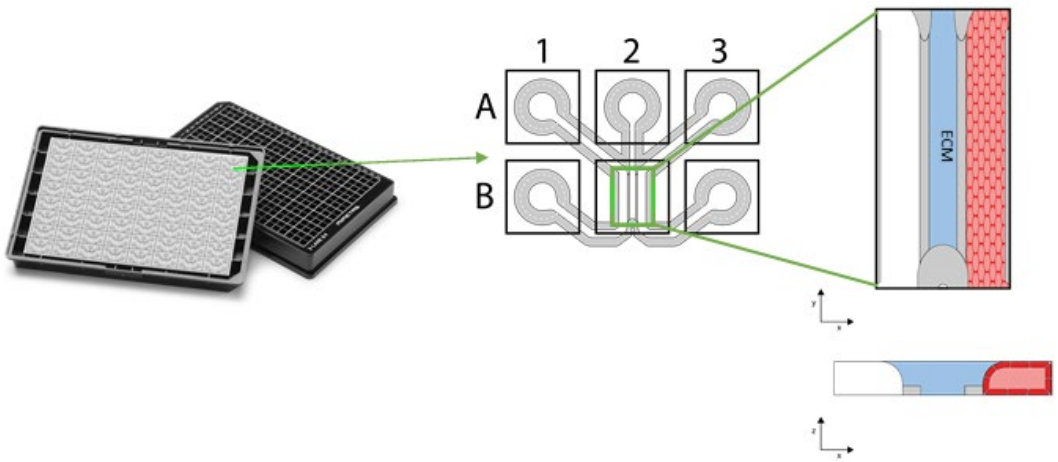

Figure 1: Endothelial tubules in the OrganoPlate® 3-lane 64. The OrganoPlate® 3-lane 64 holds 64 microfluidic chips that can be used to culture miniaturized tissue- and organ models. Each chip holds three channels. The middle channel is used to seed an extracellular matrix (ECM) gel. Endothelial cells are added to one of the perfusion channels and form an endothelial tubule under medium perfusion. The opposing perfusion channel, on the other side of the ECM gel, can be used to supply additional medium or compounds, such as a cocktail of angiogenic factors.

4. Procedures

1. Grow endothelial tubules in the right perfusion channel of an OrganoPlate® 3-lane 64
   a. See protocol *Tubule seeding – OrganoPlate® 3-lane 64*
   b. Depending on the cell type, tubule formation can take 2-5 days (~3 days for Lonza HUVEC)

2. Prepare the angiogenic cocktail in culture medium
    a. Prepare 100 µL per chip + 10% extra (±7 mL for a complete OrganoPlate® 3-lane 64)
    b. Stored at 4°C, the angiogenic cocktail should be used within one week. Prepare fresh for optimal efficiency
    c. Depending on the endothelial cell type, source, and donor, the sprouting mix composition and concentrations may require optimization. Recommended angiogenic compounds and suggested final concentrations are listed in table 1.

Table 1: Angiogenic components

Compound	Supplier	Cat. No.	Stock	Stock storage	Suggested final concencentration
rhVEGF-165	Peprotech	100-20	100 µg/mL in 0.1% BSA in PBS	-20°C	20-100 ng/mL
S1P	Sigma-Aldrich	S9666	1 mM in 95% DMSO/5% HCl 1M	-80°C	50-500 nM
PMA	Sigma-Aldrich	P1585	10 µg/mL 0.1% DMSO in MilliQ	-80°C	1-20 ng/mL
rhFGFb	Peprotech	100-18B	50 µg/mL in 0.1% BSA in PBS	-20°C	10-50 ng/mL
rhMCP-1	ImmunoTools	11343384	100 µg/mL in 0.1% BSA in PBS	-20°C	20-100 ng/mL
rhHGF	ImmunoTools	11343413	100 µg/mL 0.1% BSA in PBS	-20°C	20-100 ng/mL

3. Aspirate medium from all inlets and outlets
4. Add 50 µL medium without angiogenic factors in the inlets and outlets connected to the right perfusion channel (the channel containing the endothelial tubule)
    a. Add first to all inlets, then to all outlets to ensure that no air bubbles are trapped in channel
5. Add 50 µL angiogenic cocktail in the inlets and outlets connected to the left perfusion channel (the channel on the other side of the ECM gel, on the opposite side of the tubule)
    a. Add first to all inlets, then to all outlets to ensure that no air bubbles are trapped in channel
6. Place the OrganoPlate® back on the MIMETAS rocker platform in the incubator to continue perfusion
    a. See protocol *Tubule seeding – OrganoPlate® 3-lane 64* for correct placement on the rocker
7. Continue exposure to the angiogenic cocktail until endothelial sprouts reach the left channel
    a. Depending on the endothelial cells and the efficiency of the angiogenic cocktail used, proper sprout formation may take longer than 3 days. In such cases, it is recommended to perform a medium change after 2-3 days (refresh both regular medium and angiogenic cocktail).

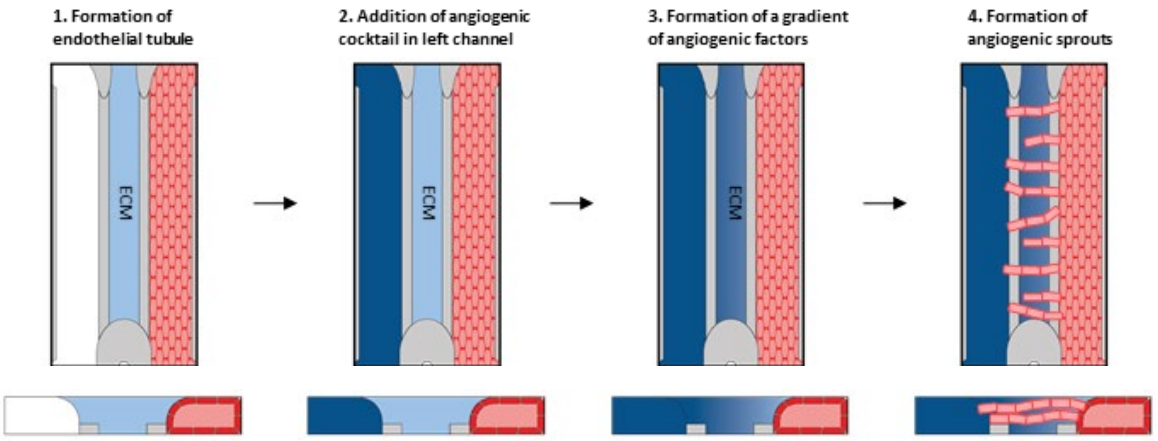

Figure 2: Formation of angiogenic sprouts in the OrganoPlate® 3-lane 64. Initially, a tubule of endothelial cells is grown in the right perfusion channel, against an ECM gel. Next, an angiogenic cocktail is added to the left perfusion channel and a gradient of angiogenic factors is formed in the ECM gel. Endothelial cells respond to the gradient by forming perfusable angiogenic sprouts that extend to the opposing 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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