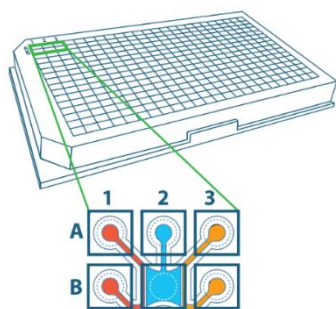


1. Objective

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 using **tip-based** liquid handlers.

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 gel chamber of the OrganoPlate® Graft **using an automated setting**.



A1 & A3: Top inlets
A2: Gel inlet
B1 & B3: Bottom outlets
B2: Graft chamber with inlet

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

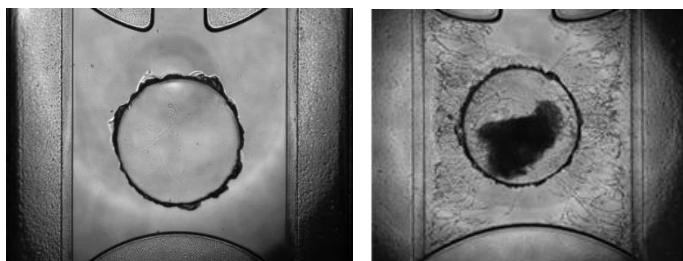


Figure 2: An ECM gel is seeded in the Graft chamber. Endothelial cells are added to the perfusion channels and form endothelial tubules (left panel). If desired, a pro-angiogenic cocktail can be applied to induce formation of a vascular network (right panel). Tissues (i.e., PDX fragments, spheroids, 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
 - The procedure for formation of a vascular network is described in the following protocol: *OrganoPlate® Graft Vascular Network Formation*
- Tissue for placement i.e., spheroids, organoids, tumor tissue, or cell aggregates
- Medium for the endothelial cells: 14 mL for all the medium in- and outlets
- Tissue-specific medium: 4mL for all the graft chambers
- Tip-based liquid handling systems
- Disposable **wide-bore** pipette tips
 - The use of wide-bore tips is highly recommended. Only with very small tissues, the regular tips might be compatible.

4. Procedures

4.1 Robotic system preparation

1. Familiarize yourself with the OrganoPlate® Graft plate and chip layout
2. Set the OrganoPlate® Graft dimensions into your robotic system (see below for OrganoPlate® Graft dimensions)
3. If necessary, test robotic system with an empty OrganoPlate® Graft

4.2 Tissue placement

This protocol describes the procedure for tissue placement that does not require ECM embedding (see figure 3).

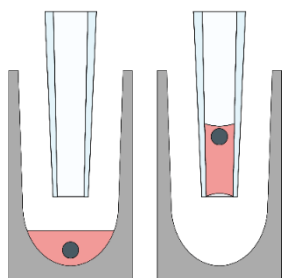
Medium change

1. Before placement of tissue, make sure all the medium in the in- and outlets and graft chambers are filled with fresh medium
 - a) Aspirate the media from all perfusion inlet and outlet wells and Graft chambers
 - b) Add 50 µL of endothelial-specific medium to the inlet and outlet well of the perfusion channels
 - c) Add 50 µL tissue-specific medium in the Graft chambers
 - *Medium change can be done with a manual pipette or with the robotic system*

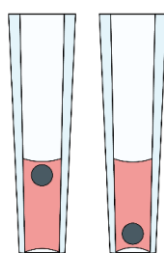
Tissue placement

1. Aspirate the tissue material in a volume of 5-50µL with the wide-bore tip
2. Allow the tissue to settle at the bottom of the pipette tip
 - The speed of the settlement is depending on the volume and the size of the tissue

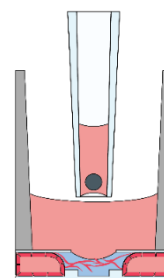
3. Position the pipette tip with the tissue in the middle of the graft chamber well. Move the pipette tip towards the graft chamber hole at a fast pace to prevent the tissue to float out of the tip before the graft chamber hole has been reached.
 - a. Make sure to not disrupt the ECM by moving the pipette tip too deep
 - b. Try to position the pipette tip right above the graft chamber hole (see OrganoPlate® Graft dimensions below for well depth)
4. When the pipette tip enters the graft chamber well, leave it there for 2-3 seconds. Allow the tissue to flow out of the wide board tip and position on the graft chamber hole
 - a. The placement of the tissue is gravity-based, make sure **not** to dispense in the graft chamber hole
5. Retract the pipette tip slowly from the graft chamber to avoid displacement of the freshly placed tissue
6. Repeat steps 2-6 for all the chips in the OrganoPlate® Graft
 - a. If a multichannel head (i.e., 8-, 96- or 384-well) is available on the robotic system, the placement of the tissues can be done using multiple tips



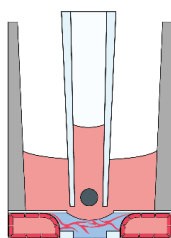
Step 1. Aspirate the tissue in a volume of 5 - 50 μ L



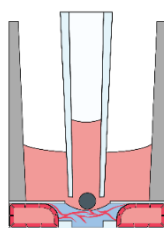
Step 2. Allow the tissue to sink to the bottom of the tip



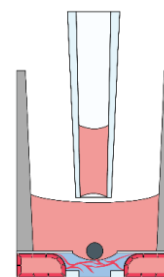
Step 3. Position the tip in the middle of the graft chamber



Step 4. Move the pipette tip towards the graft chamber hole at fast pace. Stop right above the hole



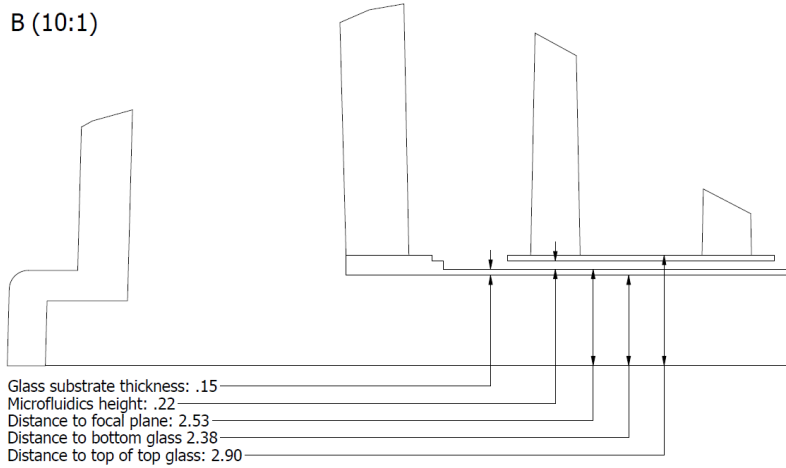
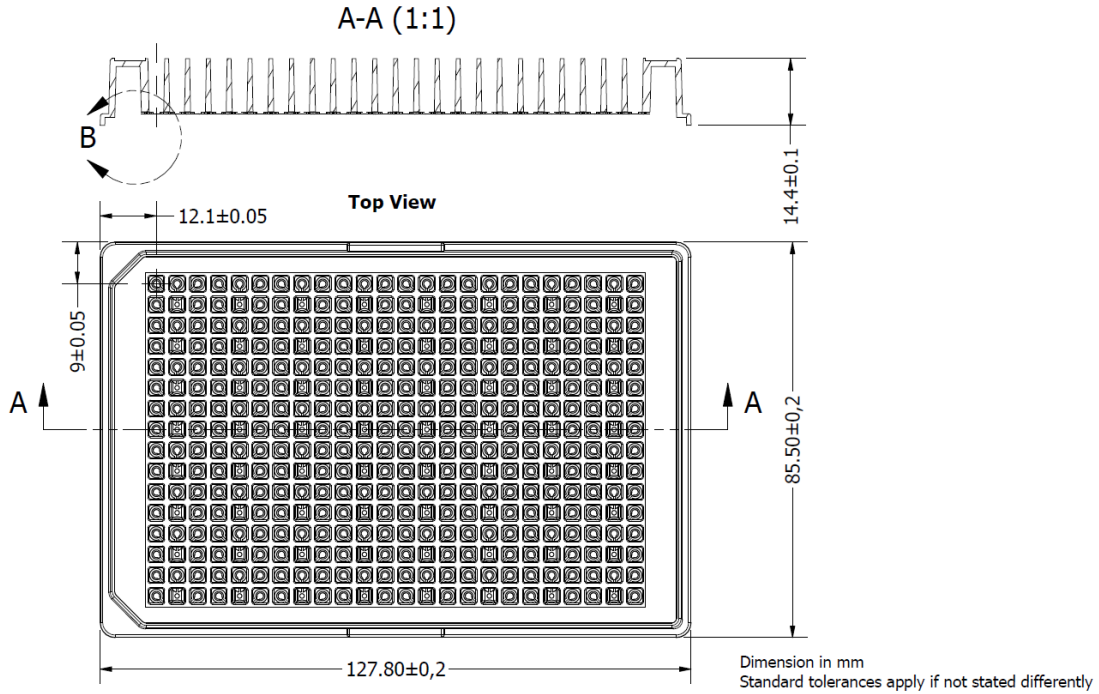
Step 5. Allow the tissue to sink onto the ECM



Step 6. Retract the pipette tip slowly from the graft chamber

Figure 3. Images describe the steps for automated tissue placement using a tip-based liquid handling system.

OrganoPlate® Graft dimensions



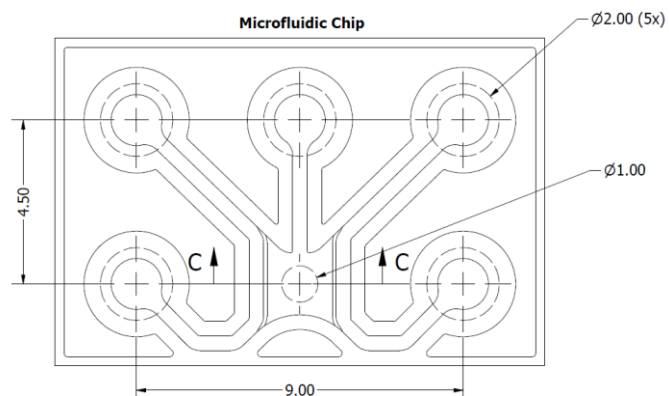


Plate layout

	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																								
B																								
C																								
D																								
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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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