Standard Operating Procedures for Dynamic Cell Culture on a Pillar Plate using a Perfusion Plate.

This standard operating procedure (SOP) provides step-by-step methods for dynamic cell culture on the 36PillarPlate/144PillarPlate using the 36PerfusionPlate/144PerfusionPlate. Please read the protocol carefully before performing experiments.

Materials:

- 36PillarPlate (Bioprinting Laboratories Inc., Cat. no. 36-01-00)
- 144PillarPlate (Bioprinting Laboratories Inc., Cat. no. 144-01-00)
- 36PerfusionPlate (Bioprinting Laboratories Inc., Cat. no. 36-02-00)
- 144PerfusionPlate (Bioprinting Laboratories Inc., Cat. no. 144-02-00)
- OrganoFlow L Digital Rocker (MIMETAS) or Infinity Rocker™ Pro (Next Advance)
- Vacusafe vacuum aspiration system (Integra Biosciences, Part no. 158320)

Methods:

1. Set the rocking parameter on the digital rocker to 10° tilting angle with 30 second frequency of tilting angle change to generate bidirectional flow in the perfusion plate (**Fig. 1**).

   Note: *The tilting angle influences the flow rate in the perfusion plate, whereas the frequency of tilting is determined by the time necessary to drain the cell growth medium in the upper reservoirs in the perfusion plate. With a higher tilting angle, a shorter frequency of tilting is required.*

Figure 1. OrganoFlow L digital rocker for loading multiple perfusion plates.

2. Dispense 600 µL of a cell growth medium in each reservoir of a 36PerfusionPlate (1200 µL cell growth medium per fluidic channel) and place it on a flat surface for 1 - 2 minutes to fill all perfusion wells with the cell growth medium (**Fig. 2**). For a 144PerfusionPlate, dispense 900 µL of a cell growth medium in each reservoir (1800 µL cell growth medium per fluidic channel). ***Note:*** *In case any perfusion wells are not filled with the cell growth medium, manually dispense 60 µL of the growth medium in the empty perfusion wells.*

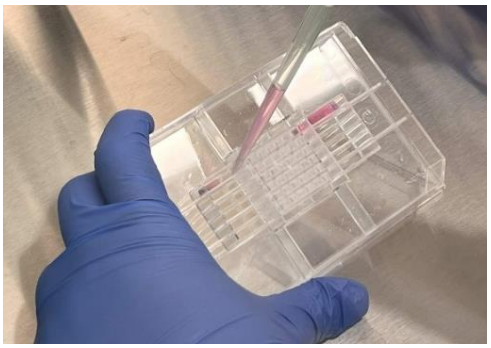

Figure 2. Manual dispensing of a cell growth medium in reservoirs of the 36PerfusionPlate.

3. Cover the 36/144PerfusionPlate with a lid and place it on the digital rocker in a 5% CO_2 incubator at 37°C for 30 minutes.
   Note: *Ensure uniform flow of the cell growth medium in the perfusion plate by checking the amount of the medium in upper and lower reservoirs as well as perfusion wells. Pre-warming the cell growth medium ensures minimal bubble formation on the 36/144PillarPlate after loading cells/spheroids in hydrogel.*
4. Take the perfusion plate out of the incubator and check the level of the cell growth medium in upper and lower reservoirs as well as perfusion wells (**Fig. 3**).

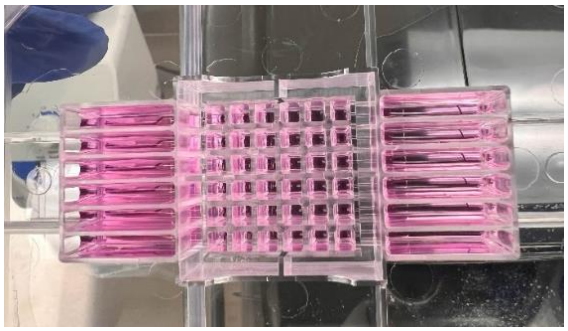

Figure 3. Uniform level of the cell growth medium in the 36PerfusionPlate.

5. Sandwich the pillar plate with cells/spheroids onto the perfusion plate and cover the sandwiched plates with a lid (**Fig. 4**).
   Note: *For cell/spheroid loading on the 36/144PillarPlate, refer to other SOPs ("Spheroid Culture on Pillar Plate", "Cell Suspension Culture in Matrigel on Pillar Plate", and "Cell Suspension Culture in Alginate on Pillar Plate").*

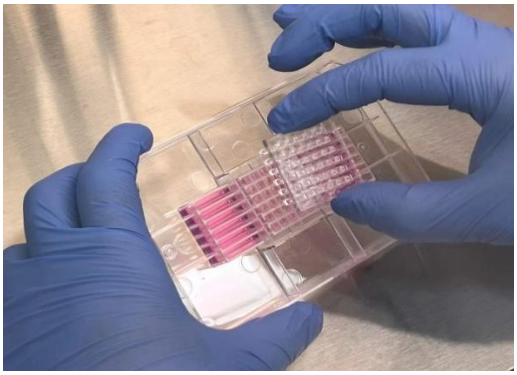

Figure 4. Sandwiching the 36PillarPlate with cells/spheroids onto the perfusion wells of the 36PerfusionPlate for dynamic cell culture.

6. Inspect the sandwiched plates under the microscope to ensure uniform cell loading on the entire pillar plate.
7. Place the sandwiched plates on the digital rocker in the 5% CO_2 incubator at 37°C and perform dynamic cell culture with medium changes every 2 - 3 days (**Fig. 5**).
   Note: *Ensure uniform flow of the cell growth medium in the perfusion plate from the sideview.*

Figure 5. Dynamic cell culture on the 144PillarPlate with the 144PerfusionPlate on the digital rocker at 10° tilting angle with a 30-second tilting angle frequency.

8. For medium change, tilt the sandwiched plates at an angle with a perfusion plate, drain the old medium into the lower reservoirs, and remove all the medium *via* vacuum aspiration from the far end of the reservoirs until no medium can be collected in the lower reservoirs (**Fig. 6**).
   Note: *Do not tilt the sandwiched plates at overly steep angles during medium change. This could lead to overflow from the lower reservoirs. It is important to remove all the old medium to avoid excessive accumulation of the cell growth medium in each fluidic channel over time. Ideally, the volume of the fresh medium added should be equal to the volume of the old medium removed.*

Figure 6. Vacuum aspiration of the old medium from the far end of the lower reservoirs.

9. Slowly dispense 600 µL of fresh, warm cell growth medium in each reservoir of the 36PerfusionPlate (1200 µL growth medium per fluidic channel). For the 144PerfusionPlate, dispense 900 µL of cell growth medium in each reservoir (1800 µL growth medium per fluidic channel).
   Note: *Be sure to add the cell growth medium from the far end of the reservoirs (far away from the microchannels) to avoid overflow in the perfusion wells by a capillary action.*
10. Place the sandwiched plates with fresh cell growth medium on the digital rocker and continue dynamic cell culture.