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

This standard operating procedure (SOP) provides step-by-step methods for dynamic cell culture on a 36PillarPlate using a 36PetriLid for 90 x 15 mm petri dishes. 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)
- 36PetriLid (Bioprinting Laboratories Inc., Cat. no. 36-03-00)
- 144PetriLid (Bioprinting Laboratories Inc., Cat. no. 144-03-00)
- Low-speed rocker (Fisher Scientific, Cat. no. 88-861-025)
- Petri dish 90 x 15 mm (VWR, Cat. no. 75799-946)

Methods:

1. For cell culture in a 90 x 15 mm petri dish, dispense 20 mL of a cell growth medium in the petri dish, cover with the 36PetriLid (for the 36PillarPlate), and place it in a 5% CO_2 incubator at 37°C for at least 1 hour to warm up the growth medium. For the 144PillarPlate, use the 144PetriLid.
   Note: *Pre-warming the cell growth medium ensures minimal bubble formation on the 36/144PillarPlate after loading cells/spheroids in hydrogel.*
2. Insert the pillar plate with cells in hydrogel in the 36PetriLid (or 144PetriLid) on the 90 x 15 mm petri dish containing 20 mL of the warm cell growth medium (**Fig. 1**).
   Note: *For cell/spheroid loading on the pillar plate, 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").*

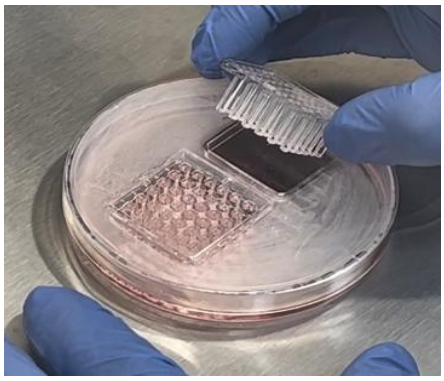
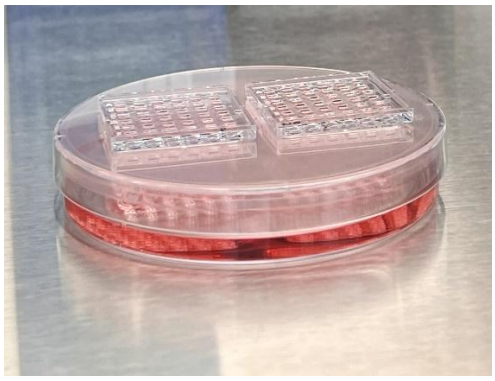
(A)  (B) 

Figure 1. (A) Inserting of the 36PillarPlate with cells/spheroids in the 36PetriLid. **(B)** Cell/spheroid culture on the pillar plate in the petri dish with a cell growth medium.

3. Inspect the pillar plates under the microscope to ensure uniform cell/spheroid loading throughout the entire pillar plate.
4. Place the pillar plate sandwiched with the petri dish on a low-speed rocker in a 5% CO_2 incubator at 37°C.
5. Set the rocking parameter of the low-speed rocker to 3° tilting angle for the 36PetriLid (2° tilting angle for the 144PetriLid) and the speed of 5 to start the rocking (**Fig. 2**).

Note: *Make sure to optimize the tilting angle and the rocking speed to prevent overflow of the cell growth medium in the petri dish. Do not use an orbital shaker for dynamic cell culture on the pillar plate. Due to physical shocks in an orbital shaker, cell spots are easily detached from the pillar plate during culture.*

6. Culture the cells/spheroids on the pillar plate in a 5% CO_2 incubator at 37°C with medium changes every 3 - 5 days.
7. For medium changes, separate the 36/144PetriLid with the pillar plate from the petri dish and sandwich it onto a 90 x 15 mm petri dish containing 20 mL of a fresh, warm cell growth medium.

Figure 2. Dynamic cell culture on the 36PillarPlate in a petri dish by rocking the cell culture medium on a low-speed rocker.