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 with a 90 x 15 mm petri dish. The shallow petri dish is used to reduce cell culture medium consumption. Please read this 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)
- Shallow 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 shallow 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: *Adding an excessive volume of cell culture medium to the petri dish can cause overflow after the pillar plate is sandwiched and the assembly is placed on a low-speed rocker. Avoid wetting the bottom of the pillars with cell culture medium, as it can lead to cross-talk or contamination during subsequent cell culture in the deep well plate or perfusion plate. Pre-warming the cell culture medium helps minimize bubble formation on the 36/144PillarPlate after loading cells or spheroids in hydrogel*
2. Insert the 36PillarPlate (or 144PillarPlate) containing cells encapsulated in hydrogel into the 36PetriLid (or 144PetriLid) placed on a 90 x 15 mm petri dish containing 20 mL of warm cell culture medium (**Fig. 1**).
   Note: *For cell/spheroid loading on the pillar plate, refer to other SOPs titled "Spheroid Culture on Pillar Plate" and "Cell Suspension Culture on Pillar Plate".*

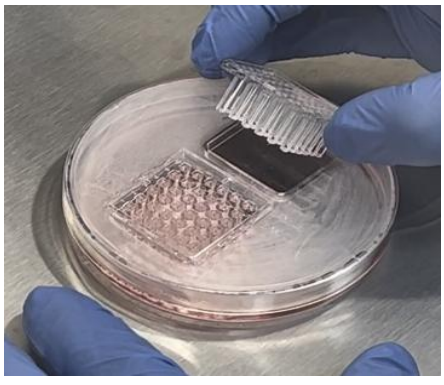
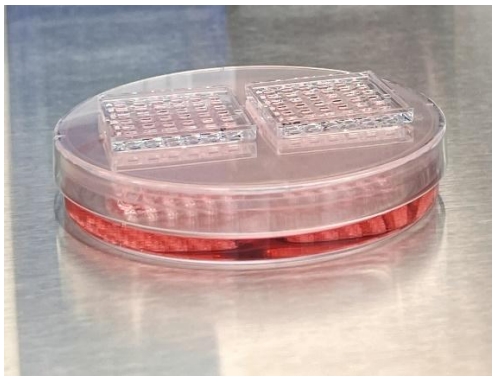
(A)  (B) 

Figure 1. (A) Insertion of the 36PillarPlate with cells/spheroids in the hole of 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 a brightfield 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 humidified 5% CO_2 incubator at 37°C.
5. Set the rocking parameters of the low-speed rocker to 3° tilt angle for the 36PetriLid (2° tilt angle for the 144PetriLid) and the speed of 5 to start the rocking (**Fig. 2**).
   Note: *Ensure that the tilt angle and rocking speed are optimized to prevent overflow of the cell culture medium in the petri dish. Do not use an orbital shaker for dynamic cell culture on the pillar plate. Due to physical shock generated by orbital shaking, cell spots can easily detach from the pillar plate during culture.*
6. Culture the cells/spheroids on the pillar plate in a humidified 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.