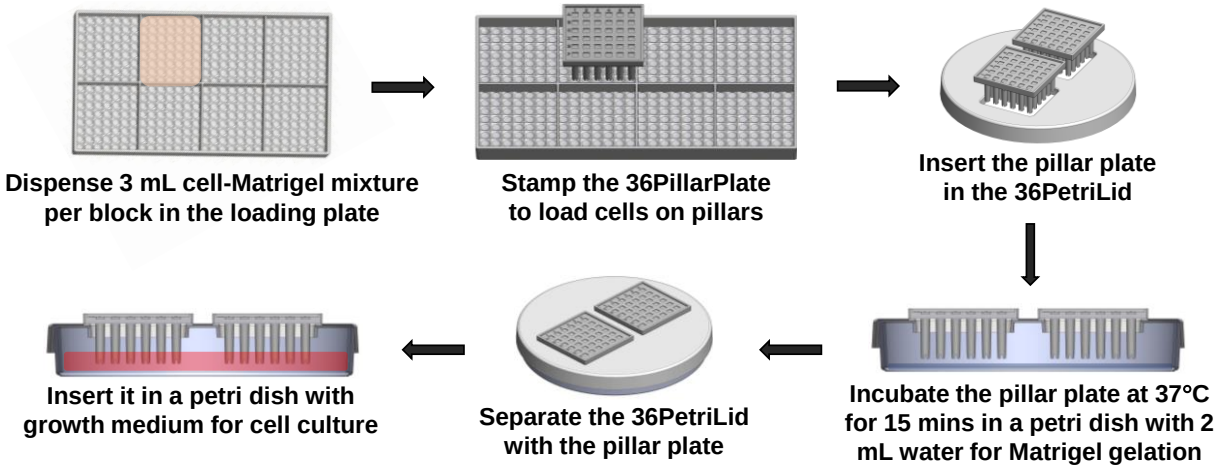
Standard Operating Procedures for Cell Suspension Culture in Matrigel on a Pillar Plate

This standard operating procedure (SOP) provides step-by-step methods for manual loading of single cell suspension in Matrigel on a 36PillarPlate and culturing cells in 3D on the 36PillarPlate with a 36PetriLid or a 384DeepWellPlate. Please read the protocol carefully before performing experiments.

Materials:

- 36PillarPlate (Bioprinting Laboratories Inc., Cat. no. 36-01-00)
- LoadingPlate (Bioprinting Laboratories Inc., Cat. no. 384-03-00)
- 36PetriLid (Bioprinting Laboratories Inc., Cat. no. 36-03-00)
- 384DeepWellPlate (Bioprinting Laboratories Inc., Cat. no. 384-02-00)
- Growth factor reduced Matrigel (Corning, Cat. no. 354230)
- Petri dish, 90 mm x 15 mm (VWR, Cat. no. 75799-946)
- Traditional 384-well plate (Fisher Scientific, Cat. no. 12-565-506)

Methods:



The overall protocol of cell suspension culture in Matrigel on the pillar plate.

Cell suspension culture in Matrigel on 36PillarPlate in petri dish or 384DeepWellPlate

Preparation of Matrigel, cell culture medium, pillar plate, and cell suspension

1. Thaw Matrigel® stock overnight by submerging the unopened bottle in an ice bucket filled with ice in a 4°C refrigerator. Prepare 500 µL aliquots of Matrigel and store at - 20°C for future use.
2. Thaw Matrigel® aliquots overnight in a 4°C refrigerator.
   Note: *It is important to thaw Matrigel aliquots in advance in a 4°C refrigerator and maintain Matrigel chilled on ice during use since Matrigel starts to solidify above 10°C. Do not freeze and thaw Matrigel aliquots.*
3. 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 lid, and place it in a 5% CO_2 incubator at 37°C for at least 1 hour to warm up the growth medium and avoid air bubble formation from the cold growth medium.
   For cell culture in the 384DeepWellPlate, dispense 80 µL/well of a cell growth medium in the 384DeepWellPlate, cover with a well plate lid, and place it in a 5% CO_2 incubator at 37°C for at

least 1 hour to warm up the medium and avoid air bubble formation.

4. Hydrate the surface of the pillar plate by inserting two 36PillarPlates in the 36PetriLid on a 90 x 15 mm petri dish containing 2 mL of sterile, distilled water and placing it in a 5% CO_2 incubator at 37°C for 20 - 30 minutes (**Fig. 1**).
   Note: *Changing the surface of the pillar plate to hydrophilic by hydration in a humid environment is necessary to minimize air bubble entrapment on the pillars after cell loading in Matrigel.*

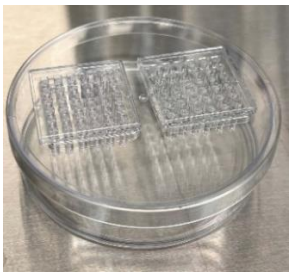


Figure 1. Hydration of the pillar plate surface in a 90 x 15 mm petri dish with 2 mL of sterile, distilled water to minimize air bubble entrapment.

5. Prepare 1.0 mL of cold cell suspension by gently mixing a cell pellet of 0.6 – 3 x 10^6 cells/pellet with 1.0 mL of a cold culture medium in a 15 mL centrifuge tube.
   Note: *The preparation of cell suspension in the cold medium is required to prevent pre-mature gelation of Matrigel.*
6. Gently mix 1.0 mL of cold cell suspension with 2.0 mL of cold Matrigel to generate a homogenous mixture of cells and Matrigel without air bubbles entrapped.
   Note: *The final cell seeding density will be 0.2 – 1 x 10^6 cells/mL in 6 – 8 mg/mL Matrigel (1,000 – 5,000 cells/pillar). Cell seeding density can be adjusted depending on the doubling time. Cells in Matrigel could settle down in 5 – 10 minutes, leading to non-uniform cell loading on the pillar plate. Keep resuspending the cell-Matrigel mixture on ice before loading in the LoadingPlate.*

Loading cell suspension in Matrigel on single pillar plate using a 1 mL pipette tip

7. Aspirate 250 µL of the cell-Matrigel mixture using a 1 mL pipette tip for single 36PillarPlate.
   Note: *It will require 5 µL of the cell-Matrigel mixture per pillar (at least 180 µL per 36PillarPlate).*
8. Separate the 1 mL pipette tip from the pipette gently to prevent cell-Matrigel spillage.
9. Using the index finger, block the back of the pipette tip (i.e., the large open area of the tip) to prevent overflow of the cell-Matrigel mixture while tapping the pillar surface.
10. Gently tap the 1 mL pipette tip with the cell-Matrigel mixture in the center of the pillar to load the cell-Matrigel mixture while blocking the large open area of the tip using the index finger (**Fig. 2**).
    Note: *Do not touch the bottom of the pillar with the pipette tip so as not to damage the surface coating. Use hydrated pillar plates in the petri dish within 5 minutes before complete water dying.*

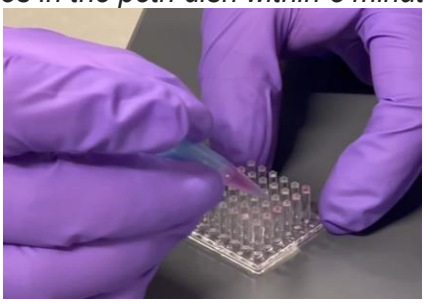


Figure 2. Loading the cell-Matrigel mixture on the pillar plate using a 1 mL pipette tip.

11. Repeat **Step 10** for all pillars.
12. After loading the cell-Matrigel mixture on all pillars, scrape excess the cell-Matrigel mixture off the pillars by sliding the 1 mL pipette tip in a horizontal position on all pillars (**Fig. 3**).

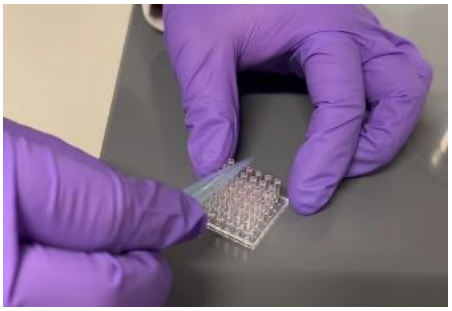


Figure 3. Scrapping excess cell-Matrigel mixture off the pillars using the 1 mL pipette tip.

Loading cell suspension in Matrigel on multiple pillar plates using a LoadingPlate

13. Place the LoadingPlate on a flat surface, dispense 2.5 - 3 mL of the cell-Matrigel mixture per small block without big bubbles, and spread it properly with the pipette tip (**Fig. 4**).
    Note: *Do not leave the cell-Matrigel mixture on the LoadingPlate for longer than 5 minutes to avoid premature gelation during stamping. Since the stamping process is quick, we don't need to place the LoadingPlate with the cell-Matrigel mixture on ice.*

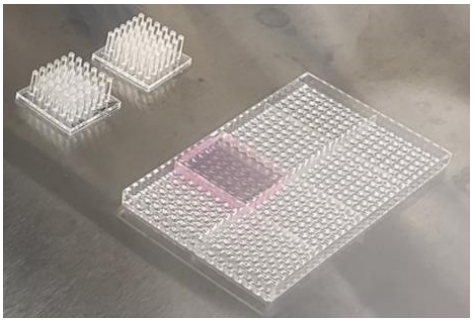


Figure 4. Dispensing 2.5 - 3 mL of the cell-Matrigel mixture per block in the LoadingPlate for rapid loading of the cells on the pillar plate.

14. Stamp the 36PillarPlate on the LoadingPlate and press gently to load the cell-Matrigel mixture evenly on the entire pillar plate (**Fig. 5**). Repeat this cell loading step for other pillar plates as needed.
    Note: *With 2.5 - 3 mL of the cell-Matrigel mixture, we can prepare at least seven 36PillarPlates (5 µL cell-Matrigel mixture per pillar or 180 µL the cell-Matrigel mixture per 36PillarPlate) without introducing macro-bubbles on the pillars. For uniform wetting of the pillars and robust cell loading, you can wiggle the pillar plate slightly during stamping.*

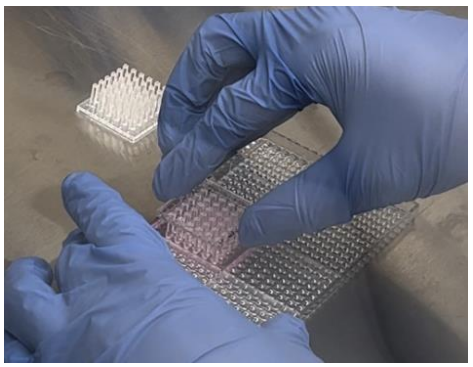


Figure 5. Stamping of the 36PillarPlate on the LoadingPlate to load cells suspended in Matrigel on pillars.

Matrigel gelation and cell culture on the pillar plate

15. Insert the pillar plate with cells in Matrigel in the 36PetriLid on a 90 x 15 mm petri dish containing 2 mL of sterile, distilled water to prevent water evaporation during Matrigel gelation (**Fig. 6**).

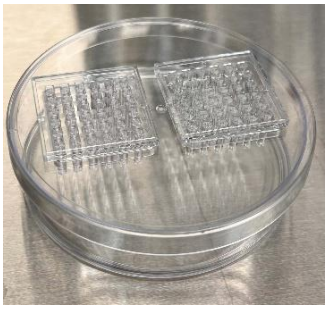


Figure 6. Gelation of Matrigel on the pillar plate inserted in the 36PetriLid on a 90 x 15 mm petri dish with 2 mL of sterile, distilled water to prevent evaporation during gelation.

16. Incubate the petri dish with the pillar plate in a 5% CO_2 incubator for 15 minutes at 37°C for complete gelation of Matrigel on the pillar plate.
17. Separate the 36PetriLid with the pillar plate and sandwich it onto the 90 x 15 mm petri dish containing 20 mL of the warm cell growth medium (**Fig. 7**) or insert the pillar plate in the 384DeepWellPlate with 80 µL/well of the warm growth medium.
    Note: *Some micro-bubbles may appear on the edge of the pillars (**Fig. 8C**), which go away in 1 - 2 days with medium change.*

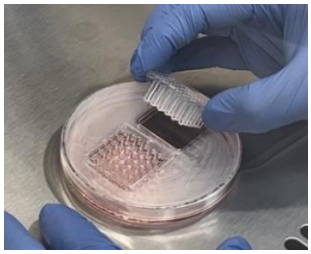
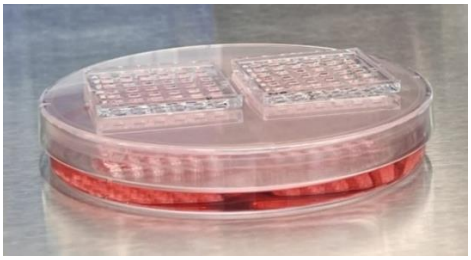
(A)  (B) 

Figure 7. (A) Inserting of the 36PillarPlate with cells in Matrigel in the 36PetriLid. **(B)** Cell suspension culture on the pillar plate in the petri dish with a cell growth medium.

18. Inspect the pillar plate under the microscope to ensure uniform cell loading throughout the entire pillar plate (**Fig. 8**).

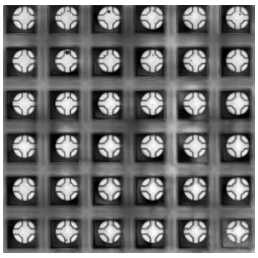
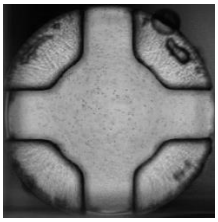
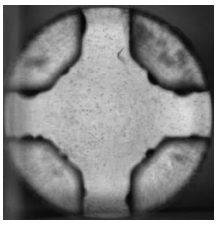
(A)  (B)  (C) 

Figure 8. (A) Stitched image of the entire 36PillarPlate with cells encapsulated in Matrigel. **(B)** Single pillar with cells in Matrigel. **(C)** Single pillar with micro-bubbles on the surface.

19. Culture the cells on the pillar plate in a 5% CO_2 incubator at 37°C with medium change every 3 - 5 days for petri dish culture or every 2 - 3 days for 384DeepWellPlate culture.
    Note: *Cells on the pillar plate in the petri dish could be cultured in a dynamic condition in a 5% CO_2 incubator with an orbital shaker/digital rocker (For dynamic 3D cell culture, refer to "Dynamic Cell Culture with PetriLid" and "Dynamic Cell Culture in Perfusion Plate").*