Bioprinting® Laboratories

Day 3: Spheroid Formation, Transfer to Pillar Plate & Culture

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Innovation in Organoid Culture and Disease Modeling

Goal: Learn experimental workflows for spheroid formation, transfer, and culture on the 36PillarPlate.

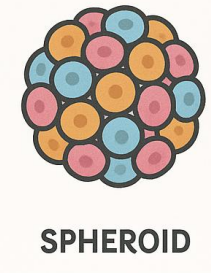
Overview:

- **Spheroid formation and transfer**
- **Optimization of cell density for spheroid formation, hydrogel viscosity, and optimum media volume**

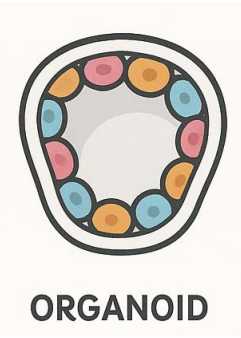
Troubleshooting common errors in spheroid transfer

3D Cell Models

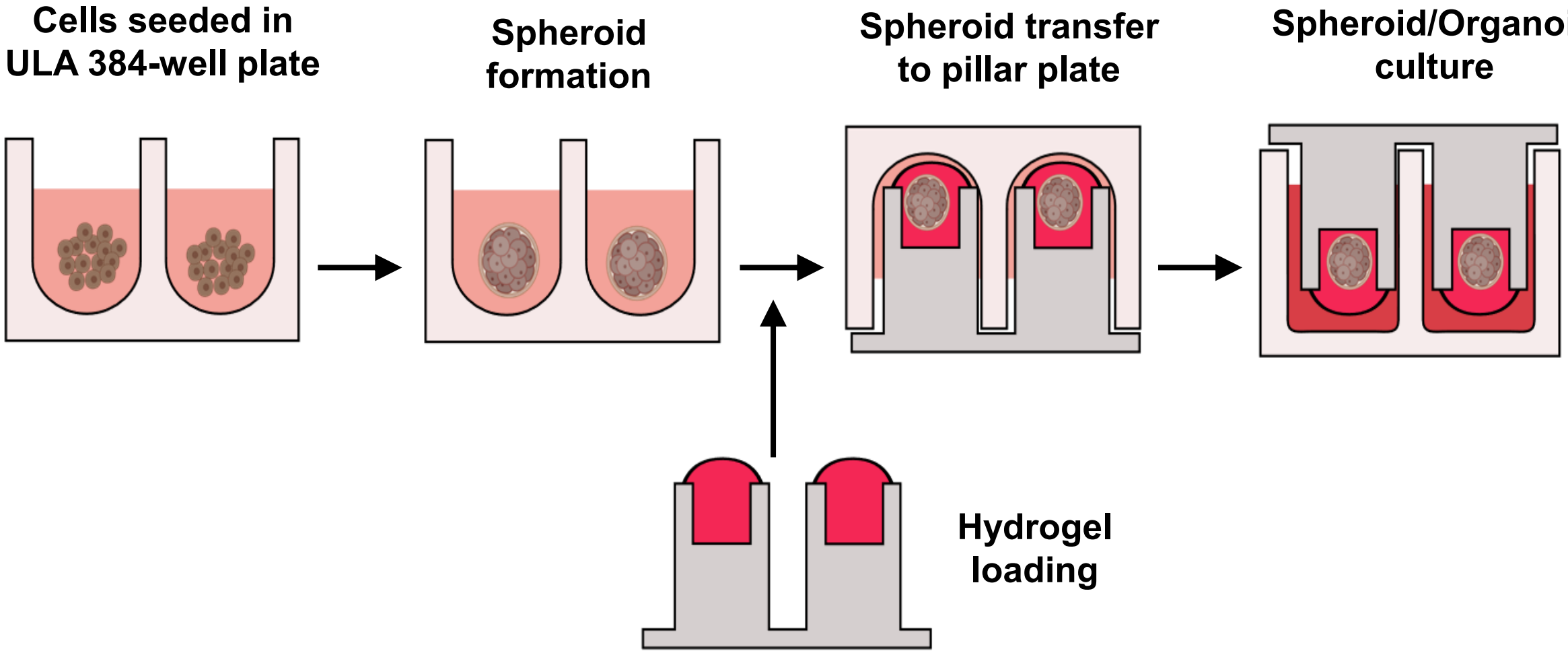
- **Spheroids**
  - **Definition:** Simple cell aggregates formed through self-assembly
  - **Applications:** Tumor modeling, drug screening and toxicity testing, and immunotherapy evaluation



- **Organoids**
  - **Definition:** Self-organized 3D structures derived from pluripotent stem cells or progenitor cells
    - Recapitulate organ-specific architecture and function (e.g., brain, liver, intestine)
    - Exhibit higher physiological relevance compared to 2D cultures
  - **Applications:** Disease modeling, precision medicine, and developmental biology



Spheroid Formation in ULA 384-Well Plates



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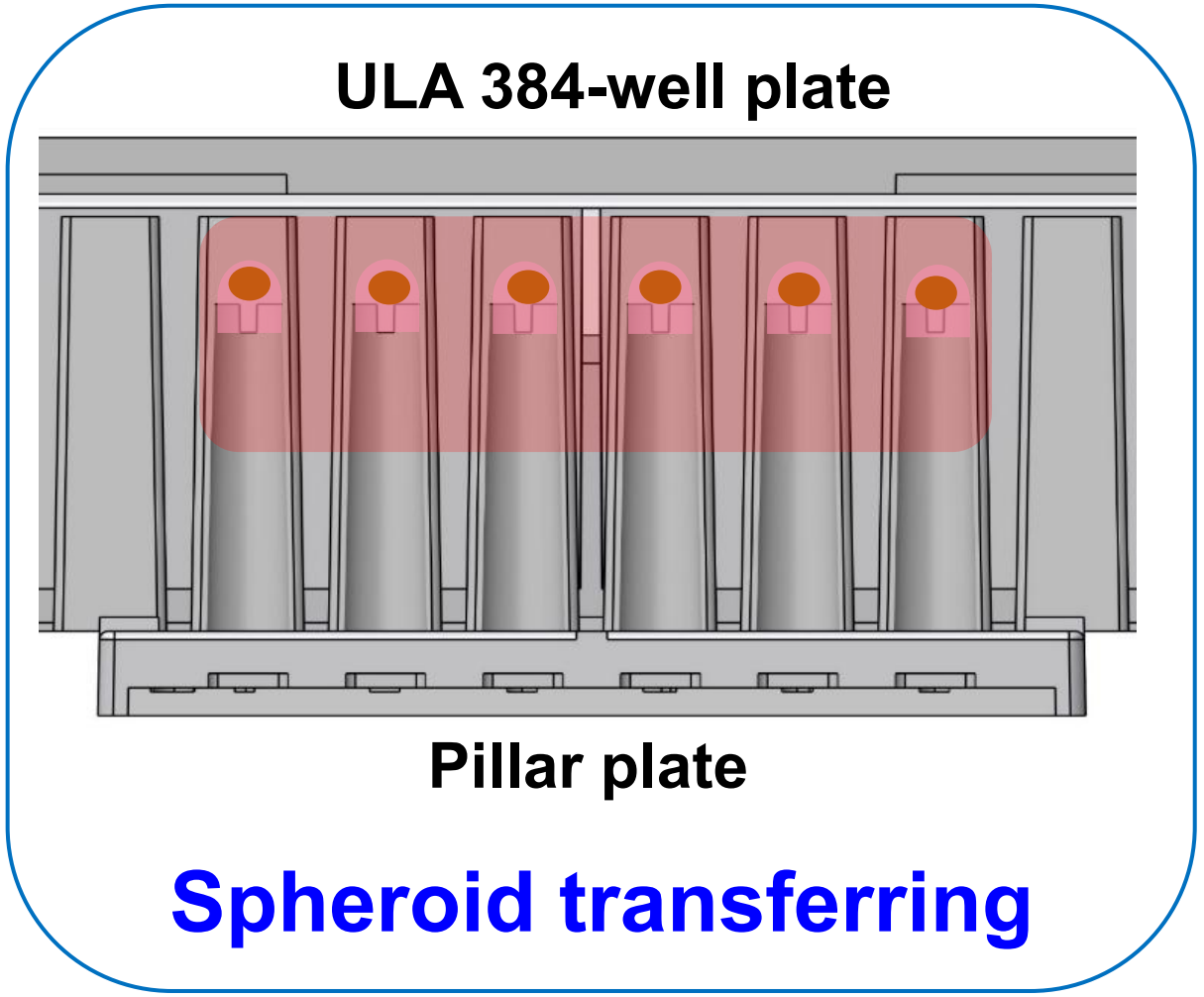
Spheroid Transfer

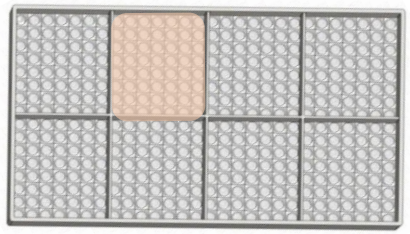
Used for the rapid transfer and encapsulation of spheroids from an ultralow attachment (ULA) 384-well plate to a pillar plate with hydrogels



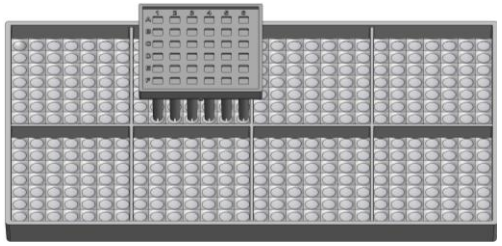
Best Practices for Spheroid Formation & Transfer

- Spheroids are formed in a ULA 384-well plate by seeding 500 - 3,000 cells per well and incubating them for 2 – 4 days.
- The typical size of cell spheroids transferred is 100 - 400 µm.
- Optimal volume of cell culture medium in each well of the ULA 384-well plate for successful spheroid transfer is 40 µL.
- An excess of medium can cause overflow during the pillar plate sandwiching process, while an insufficient volume may result in unsuccessful spheroid transfer.
- Recommended concentration of Matrigel for spheroid transfer is 6 - 8 mg/mL (i.e., 75% Matrigel).
- Recommended concentration of alginate + gelatin mixture is 0.5% alginate and 1.5% gelatin.

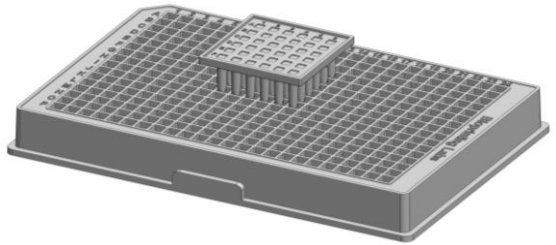
Spheroid Transfer to 36PillarPlate with Matrigel



Dispense 2 mL Matrigel per block in the loading plate

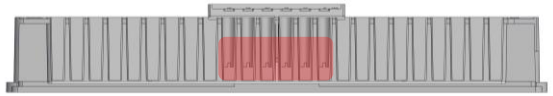


Stamp the 36PillarPlate to load Matrigel on pillars

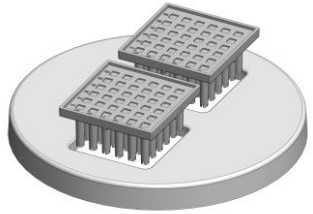


Insert the 36PillarPlate in a ULA 384-well plate with spheroids

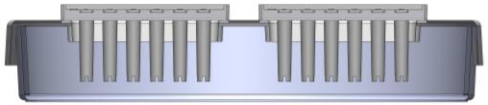
Invert the sandwiched plates and incubate at 37°C for 10 mins



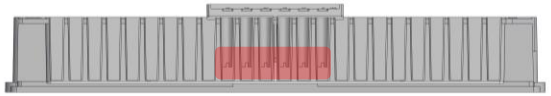
Re-invert the sandwiched plates and separate the pillar plate with spheroids in Matrigel



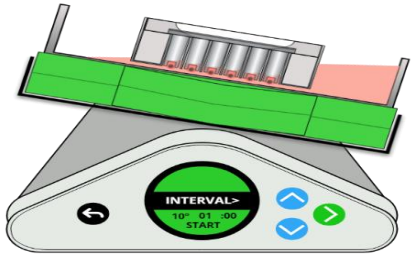
Insert the pillar plate in the 36PetriLid



Incubate the pillar plate at 37°C for 15 mins in a petri dish with 0.5 mL water for Matrigel gelation

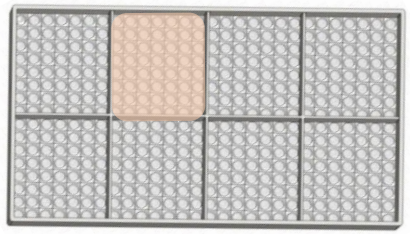


Sandwich the pillar plate onto the deep well plate for static culture

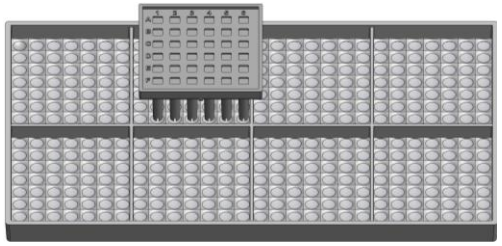


Sandwich the pillar plate onto the perfusion plate for dynamic culture

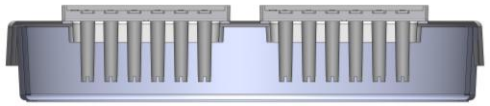
Spheroid Transfer to 36PillarPlate with Alginate-Gelatin



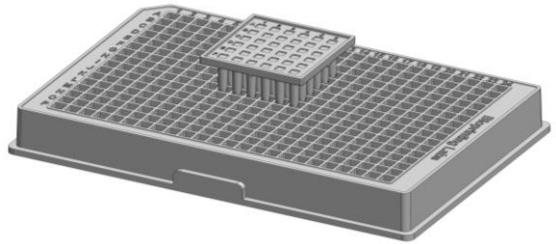
Dispense 2 mL alginate-gelatin per block in LoadingPlate



Stamp the 36PillarPlate to load alginate-gelatin on pillars

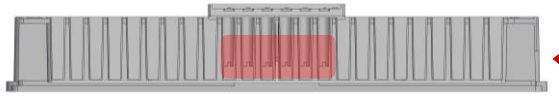


Incubate the pillar plate at 4°C for 30 mins in a petri dish

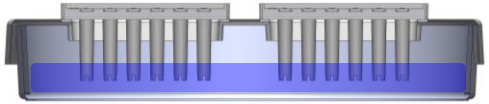


Insert the 36PillarPlate in a ULA 384-well plate with spheroids

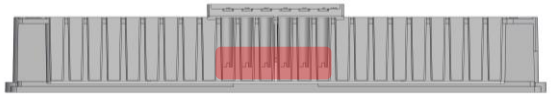
Invert the sandwiched plates and incubate at 37°C for 15 mins



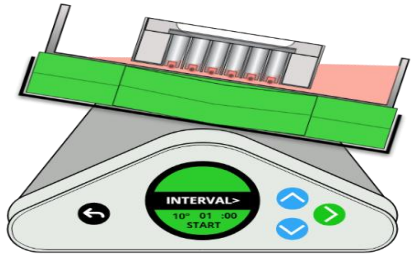
Re-invert the sandwiched plates and separate the pillar plate with spheroids in alginate-gelatin



Incubate the pillar plate at room temperature for 5 mins in a petri dish with 20 mM $CaCl_2$ + 5 mM $BaCl_2$ for alginate gelation



Sandwich the pillar plate onto the deep well plate for static culture

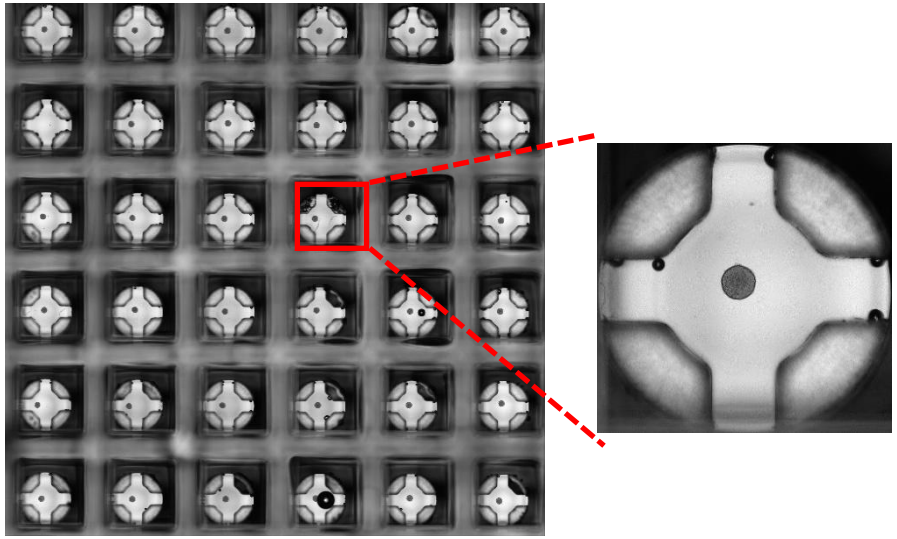


Sandwich the pillar plate onto the perfusion plate for dynamic culture

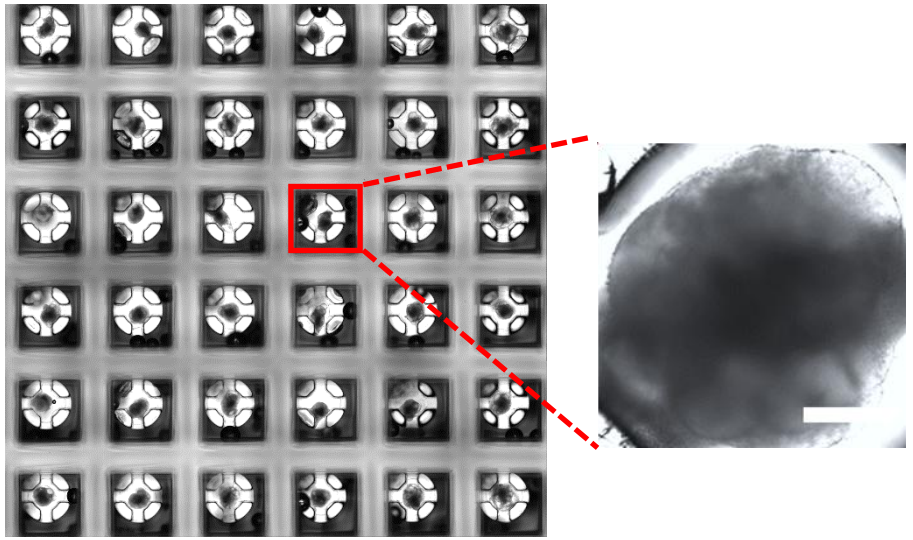
Some Precautionary Steps to Avoid Common Errors

- Thaw Matrigel aliquots in advance in a 4°C refrigerator and maintain Matrigel chilled on ice during use to avoid premature gelation of Matrigel.
- Hydrate the surface of the pillar plate in a humid environment to make it hydrophilic and minimize air bubble entrapment on the pillars after hydrogel loading.
- Centrifuge the Matrigel or alginate + gelatin mixture before loading onto the pillar plate to prevent air bubble formation.
- The 75% Matrigel loaded on the pillar plate can be pre-gelled for 2 - 3 minutes to avoid excessive dilution of Matrigel in the ULA 384-well plate during spheroid transfer.
- The pillar plate with 0.5% alginate + 1.5% gelatin should be incubated at 4°C for 30 minutes to partially solidify gelatin and minimize alginate dilution in the ULA 384-well plate during spheroid transfer

Uniformity of Organoids in the Pillar/Perfusion Plate



Liver cancer spheroids



Human brain organoids