Pillar/Perfusion Plate for Dynamic and Static 3D Cell Culture and Compound Screening

Bioprinting Laboratories Inc.

www.3dbpl.com

Innovation in Three-Dimensional (3D) Cell Culture and Disease Modeling for Non-Animal Testing

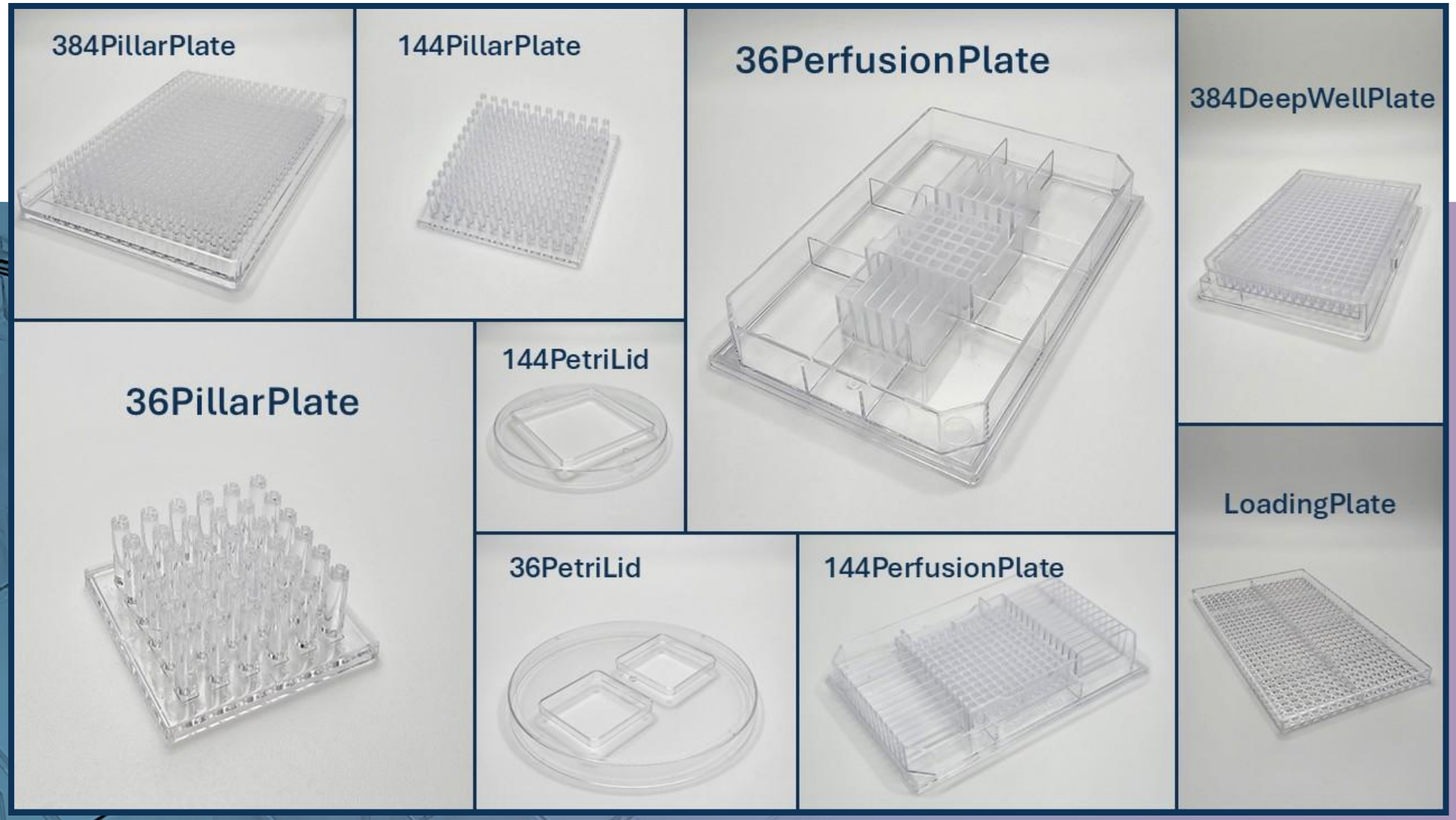
The Challenge

Traditional 2D cell cultures and simple static 3D cell models fail to replicate human physiology,
leading to costly failures in drug development.

Our Innovation

Our **Pillar/Perfusion Plate Platform** integrates **3D bioprinting, dynamic organoid culture, and high-throughput screening**, ensuring **greater accuracy, scalability, and cost effectiveness** for predictive compound testing.

Our Products: Pillar and Perfusion Plate



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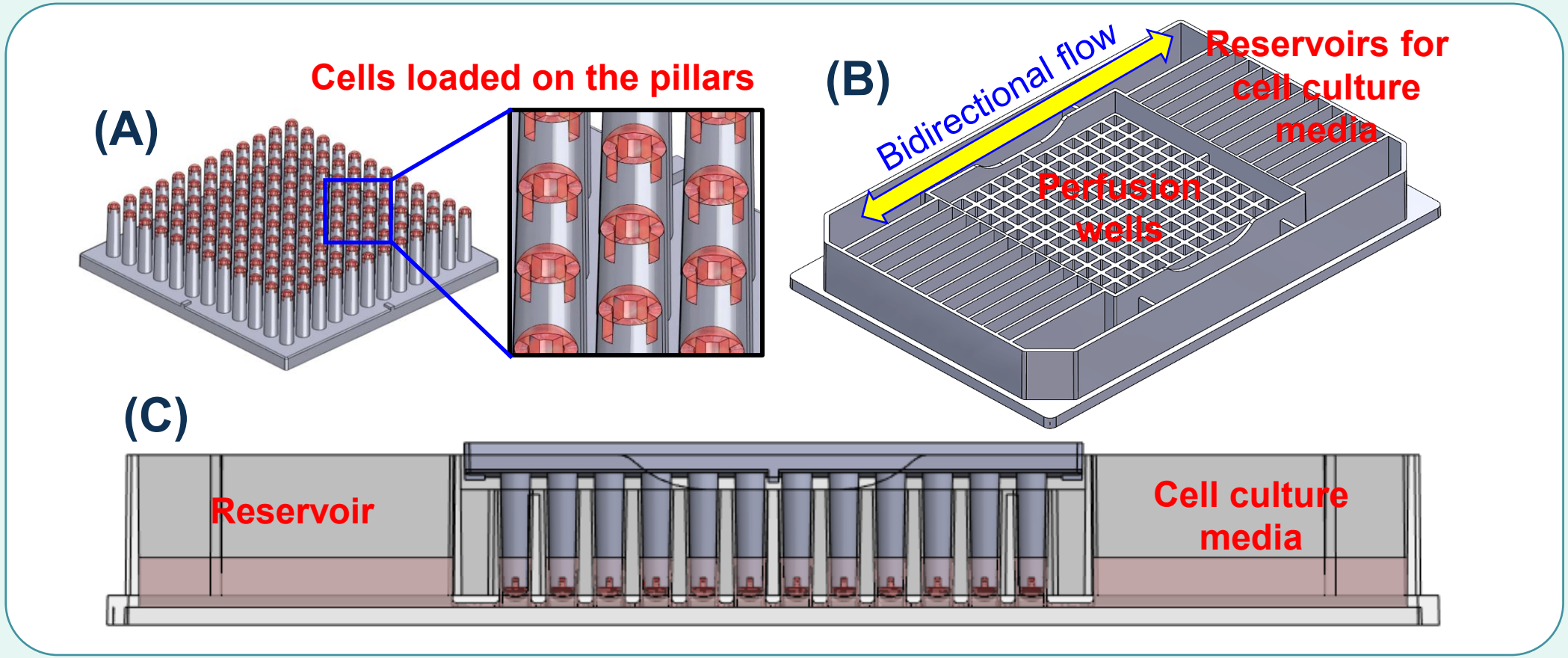
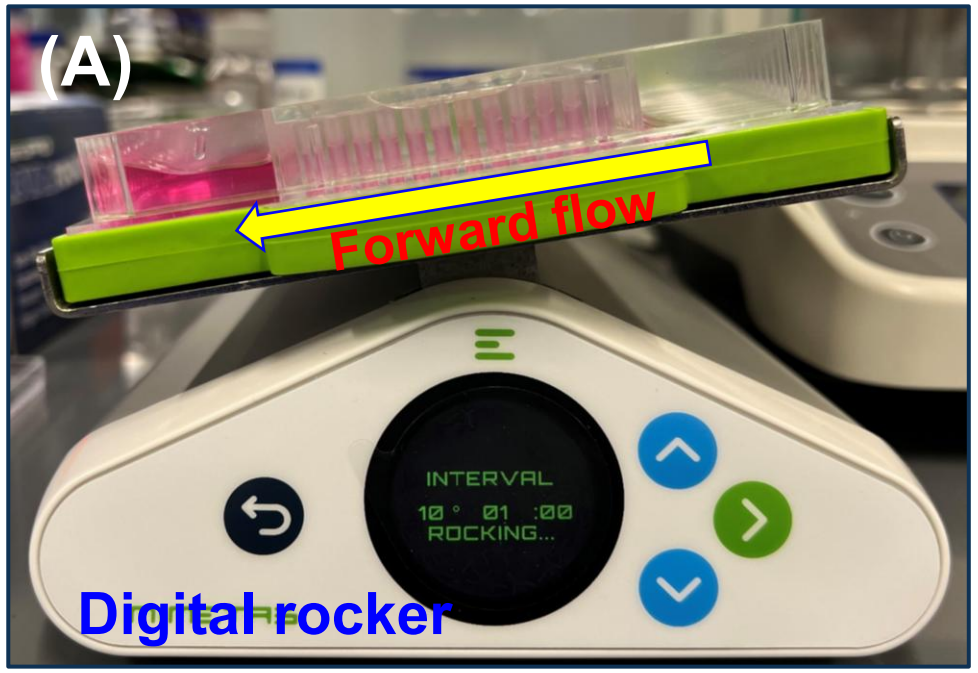
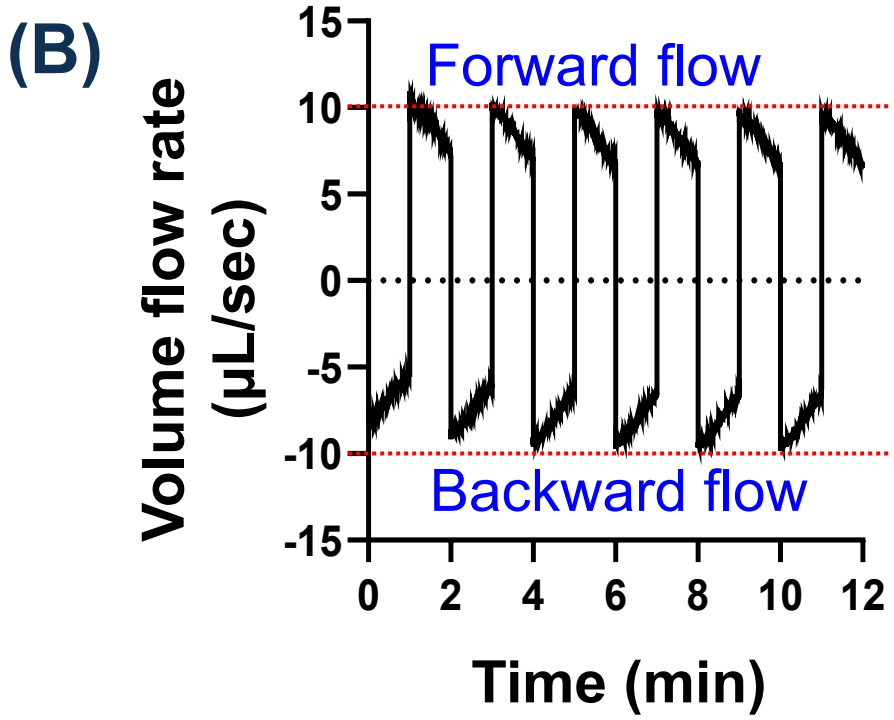
Dynamic 3D Cell Culture with Pillar/Perfusion Plate



Our 144PillarPlate and 144PerfusionPlate platform. (A) The pillar plate with a 12 x 12 array of pillars for cell loading. **(B)** The perfusion plate with a 12 x 12 arrays of perfusion wells and a 12 x 2 array of reservoirs connected by microchannels. **(C)** The pillar plate sandwiched onto the perfusion plate for dynamic cell culture.

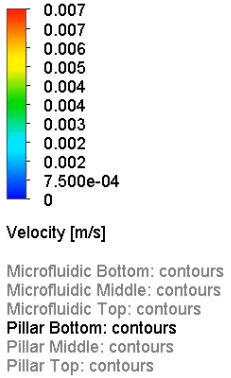
Dynamic 3D Cell Culture with Pillar/Perfusion Plate

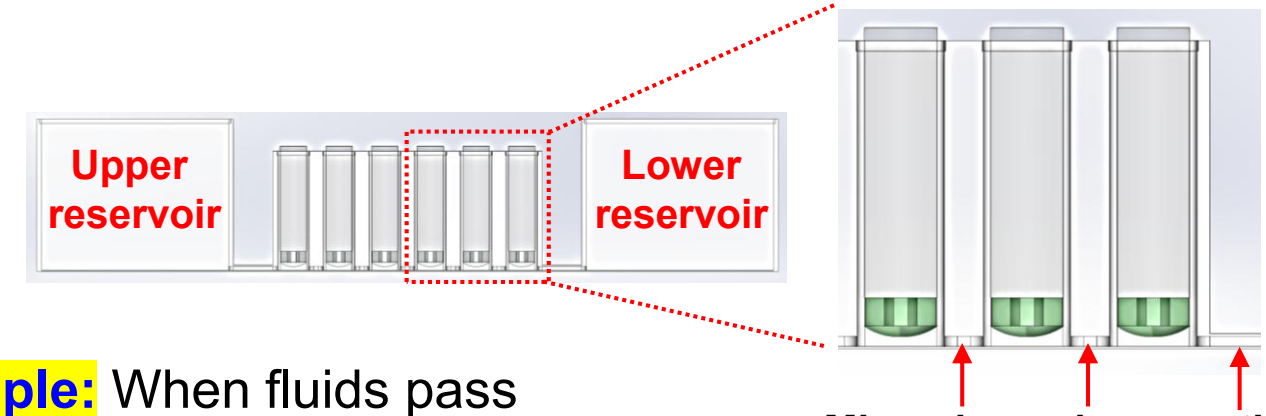




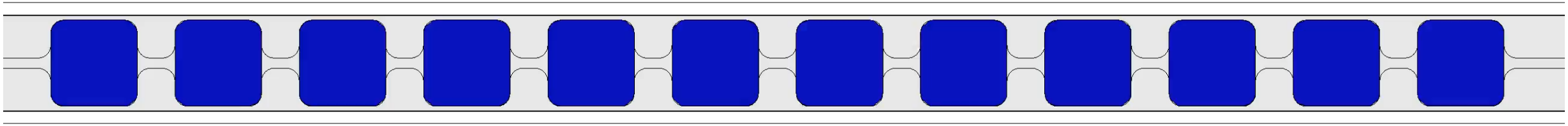
Our 144PillarPlate and 144PerfusionPlate platform. (A) The injection-molded pillar/perfusion plate on a digital rocker for dynamic organoid culture. **(B)** Flow rates in the perfusion plate with 1,500 µL culture media at 10° tilting angle and 1 minute frequency of tilting angle change. SolidWorks simulation and flow rate measurement indicate an average flow rate of 5 - 20 µL/sec, which can be adjusted by changing the tilting angle.

Velocity Profile of 144PillarPlate/144PerfusionPlate





Bernoulli's principle: When fluids pass through a narrow cross-sectional area in a pipe, flow rates are accelerated.



Velocity profiles underneath the pillars in the 144PerfusionPlate over time simulated with SolidWorks with 1500 µL of water at 10° tilting angle, 1 min frequency, and total 4 min running time. The unique design, where pillars are sandwiched into perfusion wells, facilitates rapid mixing of cell culture media, effectively minimizing diffusion limitations for nutrients and oxygen.

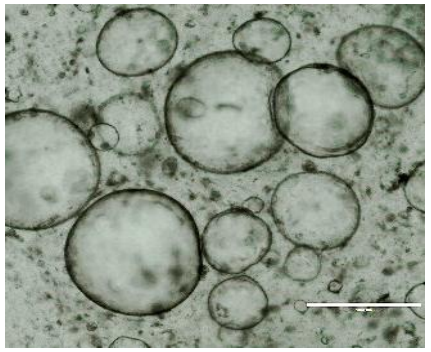
https://youtu.be/irLQIB6Ljnk

Unique Features of Our Products

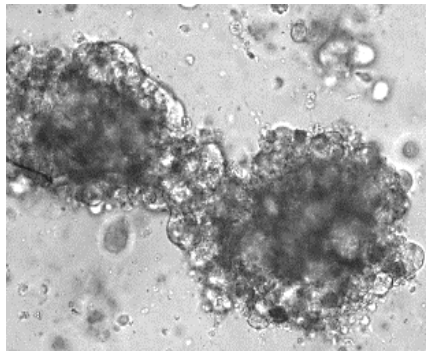
- **Manual stamping or microarray 3D bioprinting** enables rapid generation of uniform organoids on 36-, 144-, or 384-pillar arrays.
- **Pump-free, bidirectional perfusion** improves nutrient delivery and long-term organoid maturation.
- **Standard 384-well format** supports scalable production and compatibility with automated workflows (plate readers and automated microscopes).
- **10 – 100-fold miniaturization** significantly reduces assay costs.
- **On-plate cryopreservation** enables standardized organoid manufacturing, storage, and distribution.
- **Co-culture** compatible with immune, stromal, and microbial systems.
- **Injection-molded polystyrene products** minimize non-specific compound adsorption.

Human Organoids Created in the Pillar/Perfusion Plate

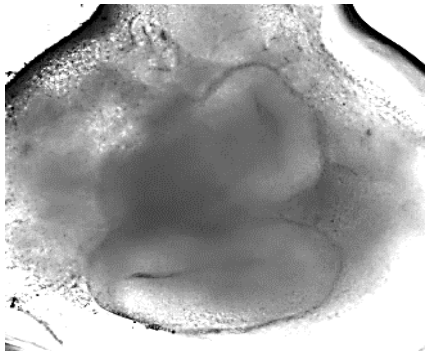
Liver



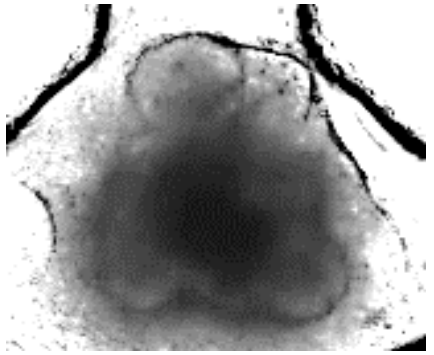
Liver tumor



Brain



Intestine



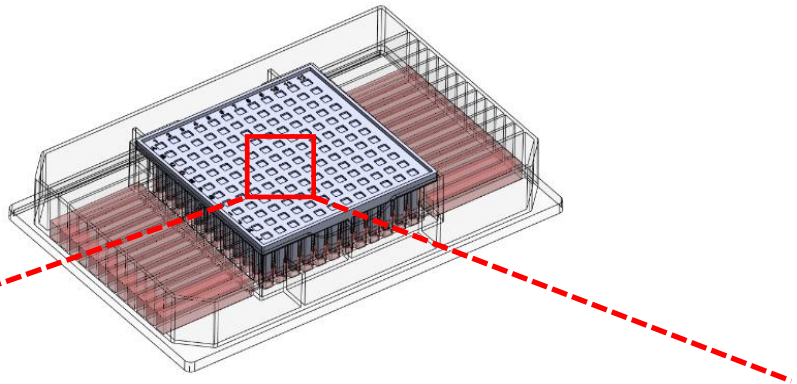
Colorectal cancer

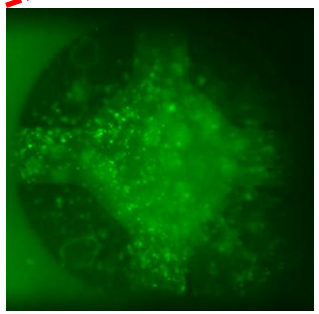
Our recent publications related to the pillar/perfusion plate products

- Kang *et al*, ***Advanced Healthcare Materials***, DOI: 10.1002/adhm.202302502 (2023)
- Acharya *et al*, ***Biofabrication***, DOI: 10.1088/1758-5090/ad1b1e (2024)
- Acharya *et al*, ***Biofabrication***, DOI: 10.1088/1758-5090/ad867e (2024)
- Shrestha *et al*, ***Lab on a Chip***, DOI: 10.1039/D4LC00149D (2024)
- Lekkala *et al*, ***ACS Biomaterials Science & Engineering***, DOI: 10.1021/acsbiomaterials.4c00179 (2024)
- Zolfaghar et al, ***ACS Biomaterials Science & Engineering***, DOI: 10.1021/acsbiomaterials.4c01383 (2024)
- Joshi *et al*, ***Toxicology In Vitro***, DOI: 10.1016/j.tiv.2023.105688 (2023)
- Shrestha *et al*, ***BioRxiv***, doi.org/10.1101/2024.03.25.586638 (2024)
- Lekkala at al, ***ACS Biomaterials Science & Engineering***, DOI: 10.1021/acsbiomaterials.4c01658 (2025)
- Joshi *et al*, ***Biotechnology and Bioengineering***, DOI: 10.1002/bit.28924 (2025)

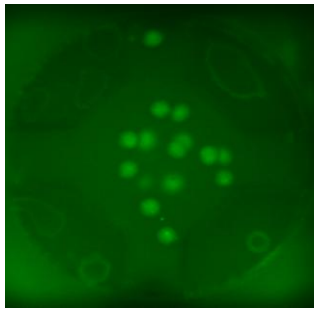
One Platform, Multiple 3D Cell Culture Applications

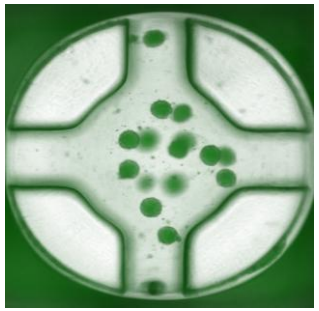
Tumor-immune cell co-culture





CellTracker Green-stained NK-92 cells



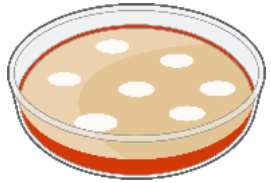


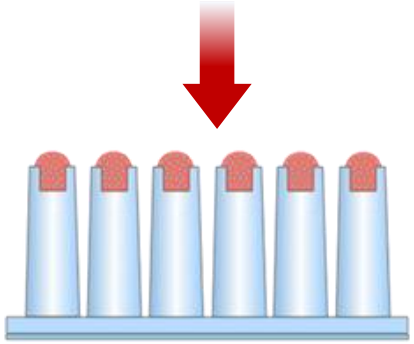
Infiltration of NK-92 cells into tumor spheroids

- Tumor-immune cell interaction studies
- Screening checkpoint inhibitors
- Immune cell-based functional assays

3D microbial cell culture

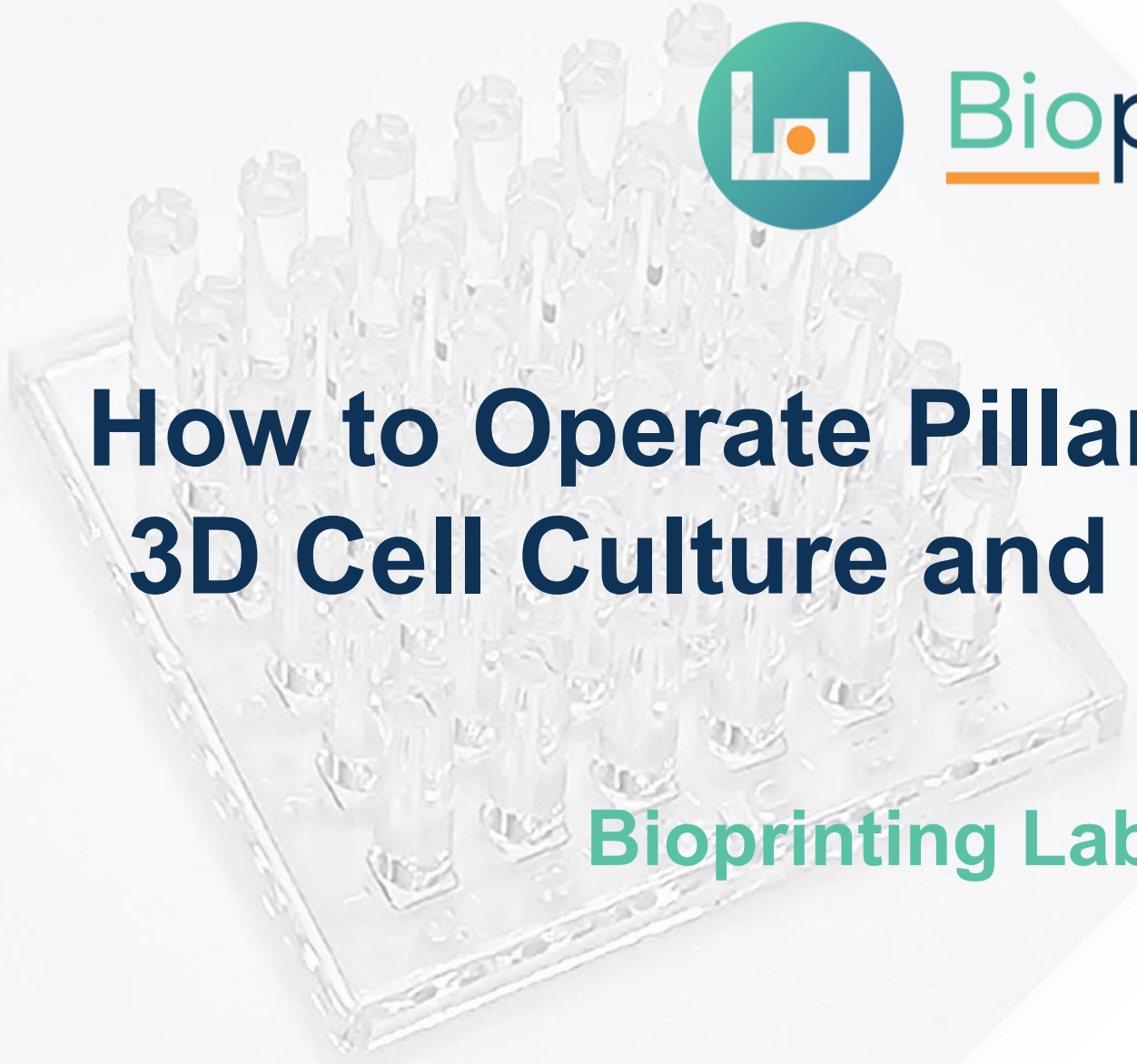
Traditional colony counting on agar plate





3D culture of microorganisms in agarose on the pillar plate

- High-throughput screening of antimicrobial compounds
- Microbial biofilm assays



How to Operate Pillar/Perfusion Plate for 3D Cell Culture and Compound Testing

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Innovation in Three-Dimensional (3D) Cell Culture and Disease Modeling for Non-Animal Testing

Essential Components for 3D Cell Culture on Pillar Plate

- **Pillar plate for cell loading and culture**
  - 36PillarPlate (36-01-00) with 36 pillars per plate, designed for both static and dynamic 3D cell culture
  - 144PillarPlate (144-01-00) with 144 pillars per plate for both static and dynamic 3D cell culture
  - 384PillarPlate (384-01-00) with 384 pillars per plate for static 3D cell culture
- **Perfusion plate for dynamic 3D cell culture and compound testing**
  - 36PerfusionPlate (36-02-00), consisting of 6 channels (each containing 6 perfusion wells and 2 reservoirs), used for dynamic 3D cell culture and compatible with the 36PillarPlate
  - 144PerfusionPlate (144-02-00), consisting of 12 channels (each containing 12 perfusion wells and 2 reservoirs), used for dynamic 3D cell culture and compatible with the 144PillarPlate
- **Deep well plate for static 3D cell culture, compound testing, and cell staining**
  - 384DeepWellPlate (384-02-00) with 384 deep wells per plate for versatile applications
- **Petri dish lid for hydrogel gelation, 3D cell culture, and cell rinsing/staining**
  - 36PetriLid (36-03-00), designed to accommodate two 36PillarPlate per petri dish
  - 144PetriLid (144-03-00), designed to accommodate one 144PillarPlate per petri dish
- **Loading plate for manual loading of hydrogel alone or single cell suspension in hydrogel on pillar plates**
  - LoadingPlate (384-03-00), designed to accommodate three types of the pillar plates for hydrogel/cell loading

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Other Components for 3D Cell Culture on Pillar Plate

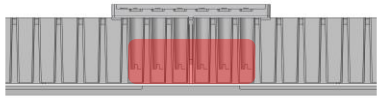
- **Hydrogels and crosslinkers for cell encapsulation on pillar plates**
  - Matrigel, growth factor reduced (Corning, 354230), used for organoid culture and primary cell culture
  - Alginic acid sodium salt, medium viscosity (Sigma Aldrich, A2033; Fisher Scientific, ICN15472480) for cancer cell culture and organoid culture in alginate-Matrigel mixtures
    - A 2% (w/v) medium-viscosity alginate solution was prepared by dissolving 200 mg of alginic acid sodium salt in 10 mL of sterile distilled water under continuous stirring for 3 days, followed by filtration and storage at 4°C until use.
  - Agarose, low temperature gelling (Sigma Aldrich, A2576) for 3D microbial culture
  - Calcium chloride (Sigma Aldrich, C7902), used for alginate gelation
  - Barium chloride (Sigma Aldrich, B0750), used for strong alginate gelation to minimize alginate degradation by phosphate ions in cell culture media and buffer solutions
- **Additional devices**
  - Deep petri dish, 100 mm x 20 mm (Corning, 70165-102), used in combination with the Petri Lid for pillar plate hydration and hydrogel gelation
  - Traditional 384-well plate (Fisher Scientific, 12-565-506), used for cell staining and imaging on pillar plates
  - Ultralow attachment (ULA) 384-well plate (S-BIO, MS-9384UZ; FaCellitate, F224384), used for spheroid formation
- **Dynamic cell culture equipment**
  - OrganoFlow L digital rocker (MIMETAS) for dynamic 3D cell culture with perfusion plates
  - Low-speed rocker (Fisher Scientific, Cat. no. 88-861-025) for dynamic 3D cell culture with petri dishes

Selection Guide for Pillar/Perfusion Plate Products

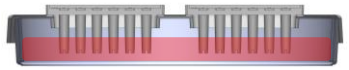
Dynamic 3D cell culture necessary?

NO → **Multiple culture/test conditions?**

- **YES** →
  - **36PillarPlate** (or **144PillarPlate**)
  - **384DeepWellPlate**
  - **36PetriLid** (or **144PetriLid**)
  - **LoadingPlate**

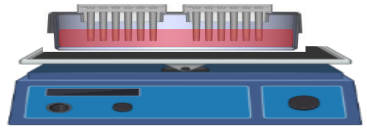


- **NO** →
  - **36PillarPlate** (or **144PillarPlate**)
  - **Petri dish**
  - **36PetriLid** (or **144PetriLid**)
  - **LoadingPlate**



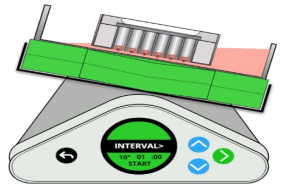
YES → **Multiple culture/test conditions?**

- **NO** →
  - **36PillarPlate** (or **144PillarPlate**)
  - **Petri dish**
  - **36PetriLid** (or **144PetriLid**)
  - **LoadingPlate**
  - **Low-speed rocker**



Orbital flow

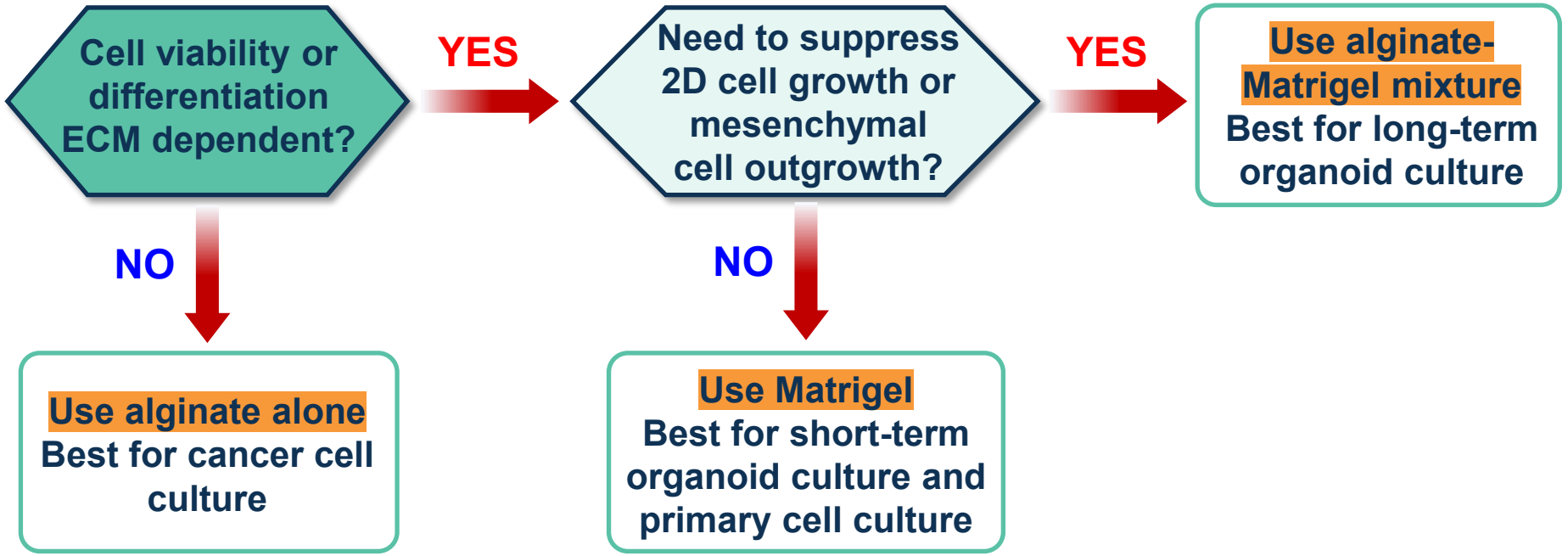
- **YES** →
  - **36PillarPlate** (or **144PillarPlate**)
  - **36PerfusionPlate** (or **144PerfusionPlate**)
  - **36PetriLid** (or **144PetriLid**)
  - **LoadingPlate**
  - **Digital rocker**



Bidirectional flow

Use the 384PillarPlate for high-throughput, 3D microbial assays.

Selection Guide for Hydrogels for Cell Encapsulation

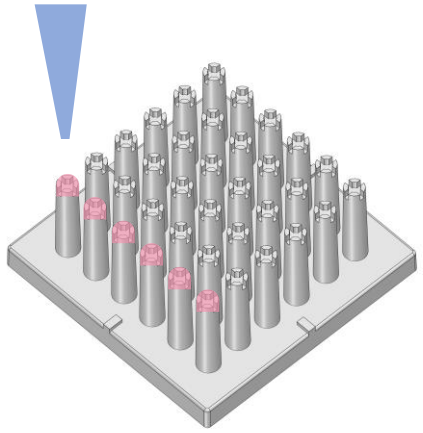


- **For Matrigel gelation,** the 36PillarPlate containing cells in Matrigel was inserted into the 36PetriLid and placed on a 100 × 20 mm petri dish containing 5 mL of sterile distilled water to minimize evaporation. The petri dish assembly was then incubated in a humidified 5% CO_2 incubator at 37°C for 15 - 20 minutes.
- **For alginate gelation,** the 36PillarPlate containing cells in alginate was inserted into the 36PetriLid and placed on a 100 × 20 mm petri dish containing 60 mL of 0.9% NaCl supplemented with 20 mM $CaCl_2$ and 5 mM $BaCl_2$. The assembly was incubated at room temperature for 5 minutes for gelation, followed by rinsing in 0.9% NaCl for 5 minutes.

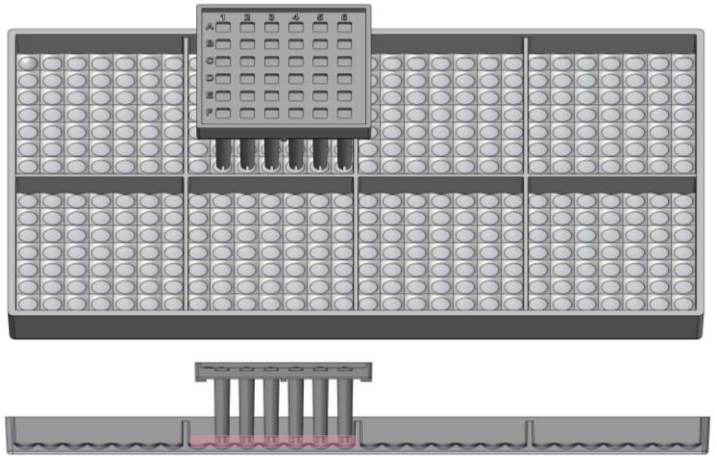
Hydrogels Used and Gelation Methods

Cell preparation	Final hydrogel concentrations	Loading methods	Hydrogel gelation methods	Spheroid transfer time	Rinsing methods
Single cell suspension	75% Matrigel	Pillar plate stamping	15-minute incubation at 37°C in a petri dish	-	-
Single cell suspension	0.5% alginate + 50% Matrigel	Pillar plate stamping	15-minute incubation at 37°C, followed by 5-minute incubation in 0.9% NaCl containing 20 mM $CaCl_2$ and 5 mM $BaCl_2$	-	5-minute incubation in 0.9% NaCl
Spheroids in ultralow attachment (ULA) 384-well plate	50% Matrigel	Pillar plate stamping + spheroid transfer	20-minute incubation at 37°C in a petri dish	4 hours	-
Spheroids in ultralow attachment (ULA) 384-well plate	0.5% alginate + 50% Matrigel	Pillar plate stamping + spheroid transfer	20-minute incubation at 37°C, followed by 5-minute incubation in 20 mM $CaCl_2$ and 5 mM $BaCl_2$	4 hours	5-minute incubation in 0.9% NaCl

Rapid and Robust Cell Loading on Pillar Plate

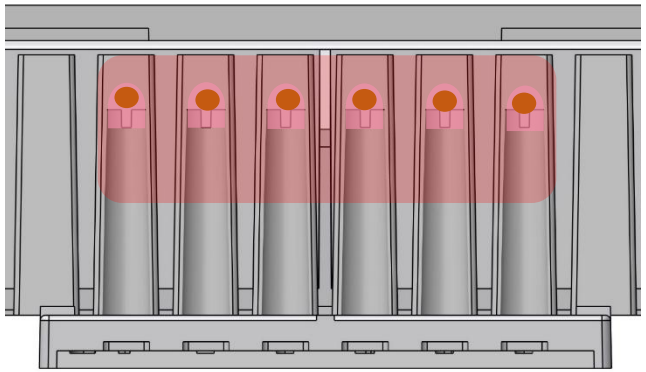


Manual dispensing with 1 mL pipette tip



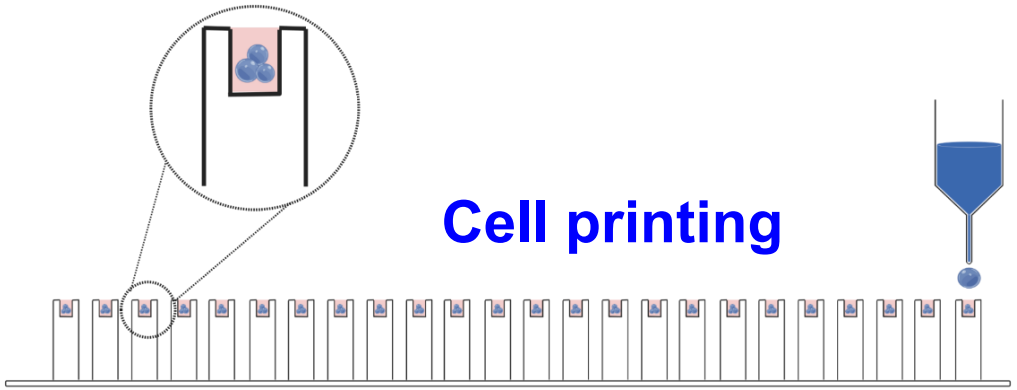
Pillar plate stamping

ULA 384-well plate



Spheroid transferring

Pillar plate



Cell printing

https://youtu.be/EaDw4xoNuel

Selection Guide for Cell Loading Methods

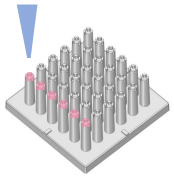
Starting with single cells or spheroids for 3D cell culture?

Single cells encapsulated in hydrogel

Multiple pillar plates prepared?

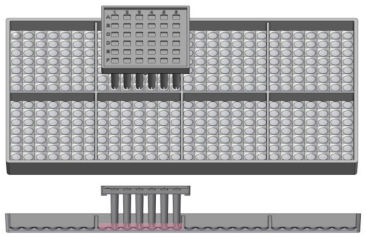
NO

Manual dispensing



YES

Pillar plate stamping



Spheroids or cell aggregates attached on hydrogel

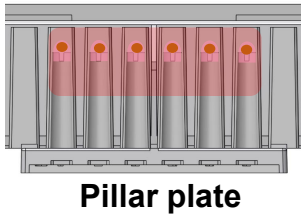
Multiple pillar plates prepared?

NO

Manual dispensing or spheroid transfer

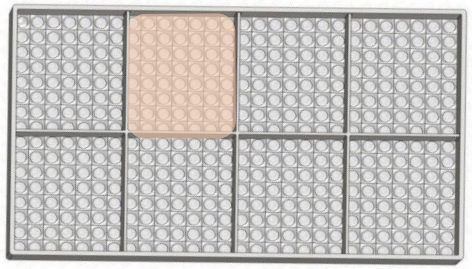
YES

Spheroid transfer

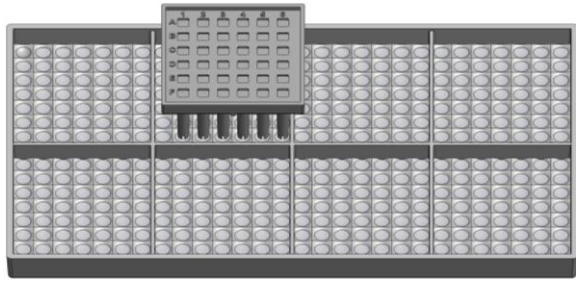


Bioprinting single cells or spheroids suspended in hydrogels onto pillar plates enables scalable production of 3D cell cultures.

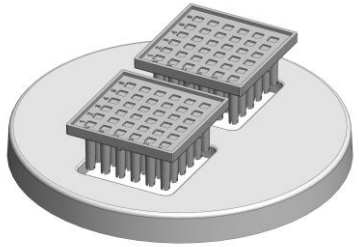
Pillar Plate Stamping for Rapid and Robust Cell Loading



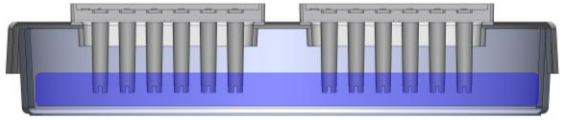
Dispense single cells suspended in alginate in the LoadingPlate



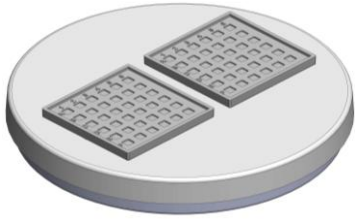
Stamp the 36PillarPlate to load cells on pillars



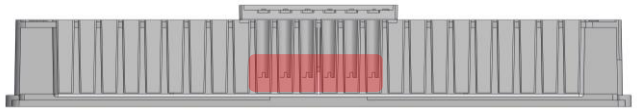
Insert the pillar plate in the 36PetriLid



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



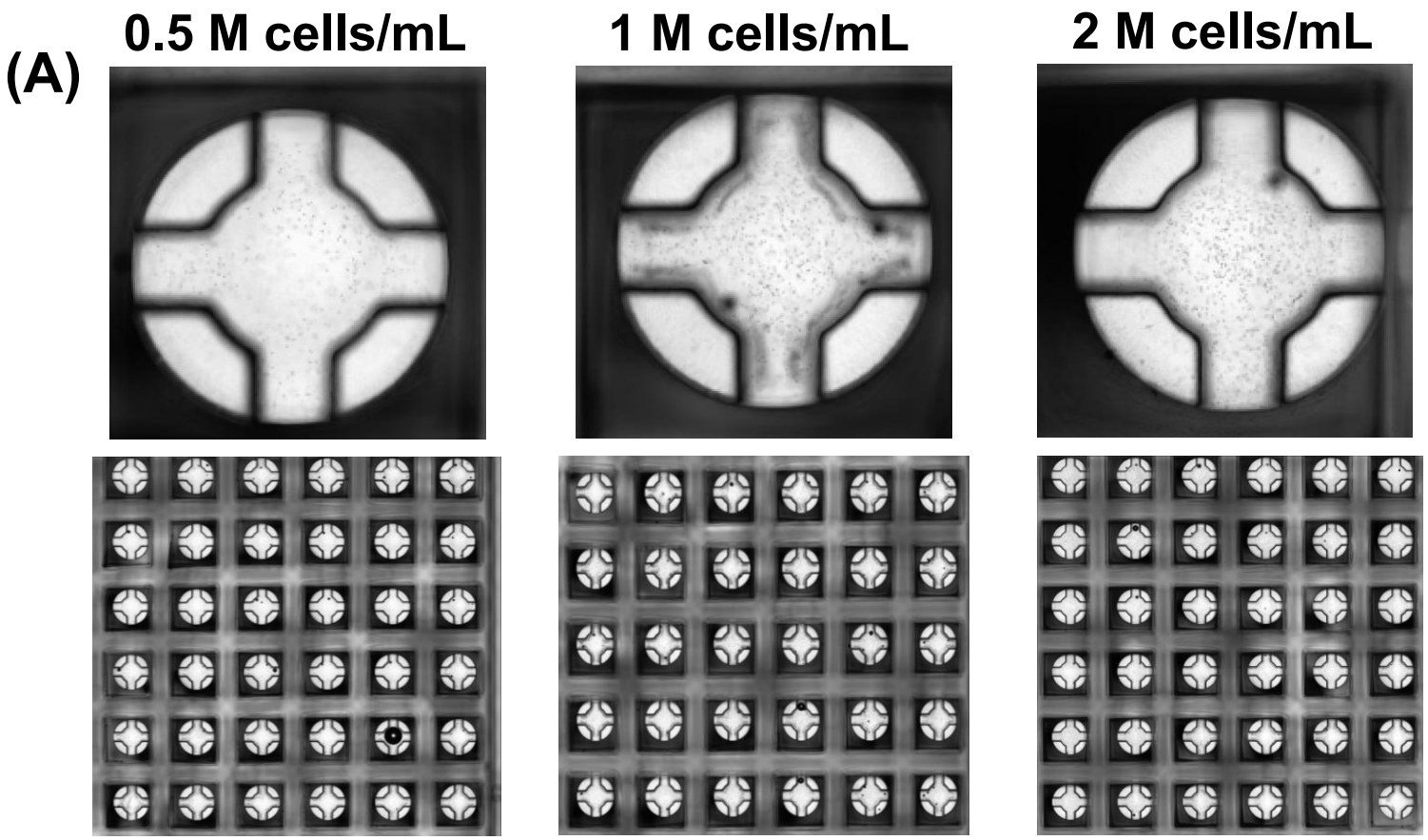
Separate the pillar plate from the 36PetriLid and rinse it to remove excess salts

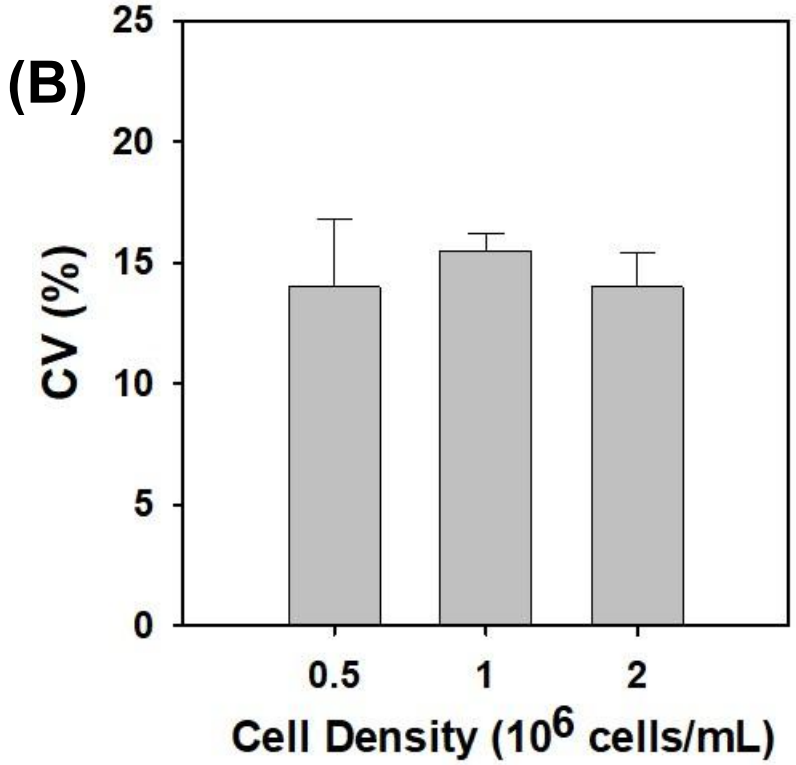


Sandwich the pillar plate onto the deep well plate with growth medium for cell culture

Manual stamping of the pillar plate into cells suspended in alginate for static 3D cell culture in a deep well plate.

Reproducibility of Single Cell Loading by Pillar Stamping

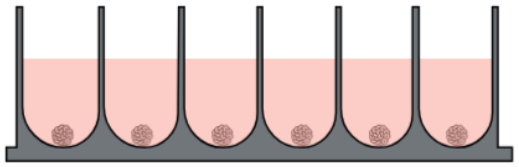




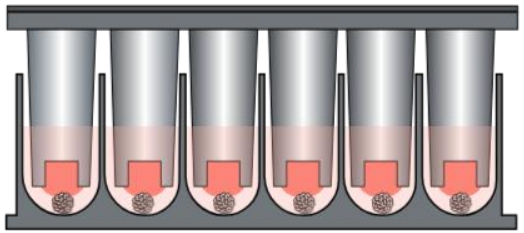
DOI: 10.1002/bit.28924

(A) Hep3B cells suspended in 0.75% alginate loaded on the 144PillarPlate by manual stamping using the LoadingPlate. Three cell seeding densities (0.5, 1, and 2 x 10^6 cells/mL) were prepared and added in the LoadingPlate (1.5 mL/well) for stamping. Alginate on the 144PillarPlate formed a gel in 15 mM $CaCl_2$ using the 144PetriLid for 5 min. **(B)** Coefficient of variation (CV) measured by cell viability with CellTiter-Glo assay. CV values remain below 15%, demonstrating high reproducibility in cell loading.

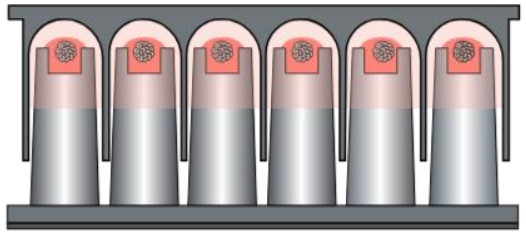
Spheroid Transfer from ULA Well Plate to Pillar Plate



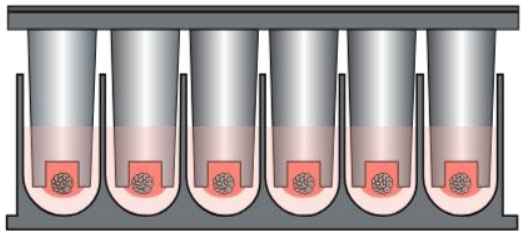
Create spheroids
in an ULA 384-well plate



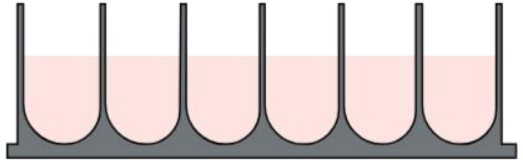
Sandwich the pillar plate with
Matrigel onto the ULA plate



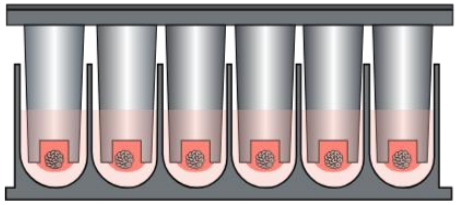
Invert the sandwiched plates
and incubate for 4 hours



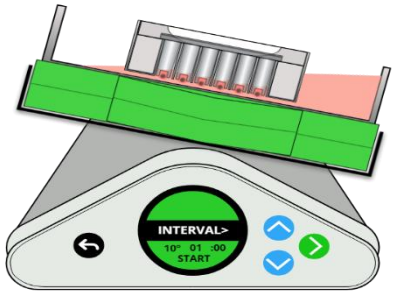
Re-invert the sandwiched plates
with the pillar plate on top



Separate the pillar plate
from the ULA plate

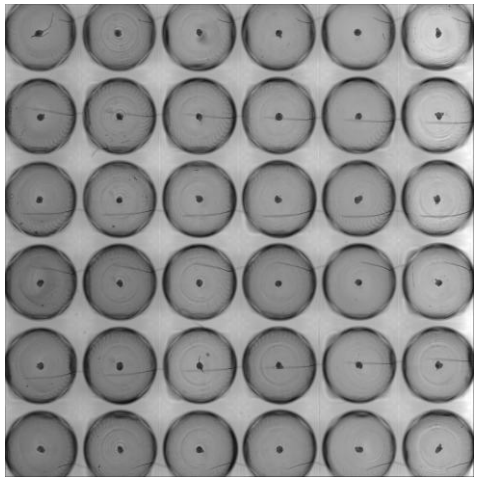


Sandwich the pillar plate onto the
<u>deep well plate</u> (**Static culture**)



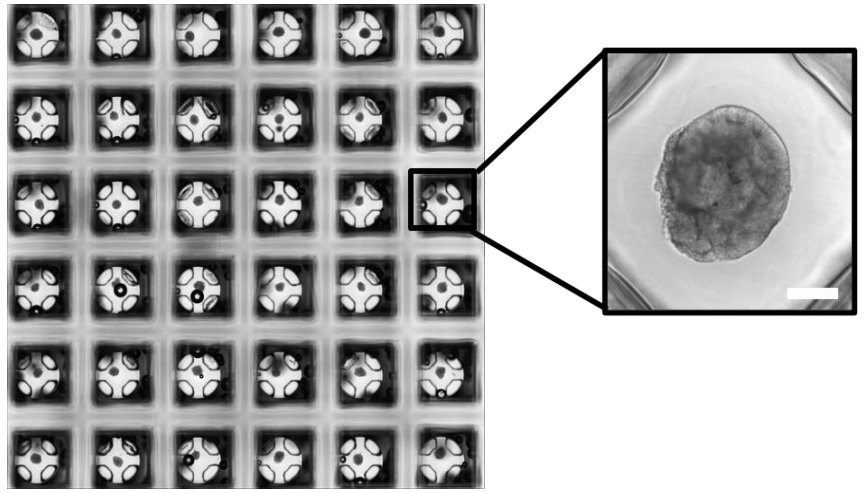
Sandwich the pillar plate onto the
<u>perfusion plate</u> (**Dynamic culture**)

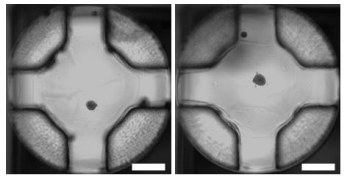
Fast and Simple Spheroid Loading on Pillar Plate



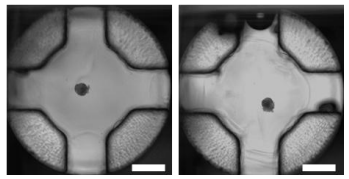
ULA 384-well plate with spheroids

Spheroid transfer to the pillar plate

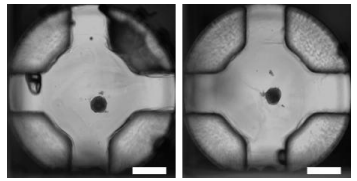




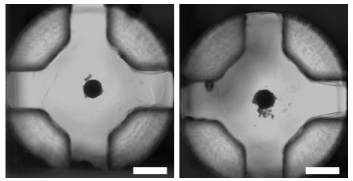
500 cells/well



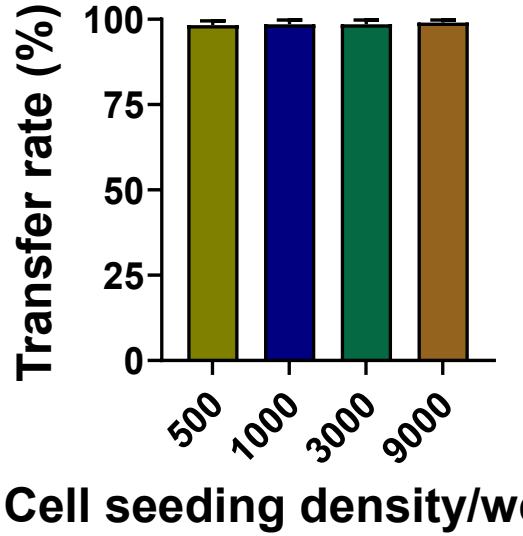
1000 cells/well



3000 cells/well

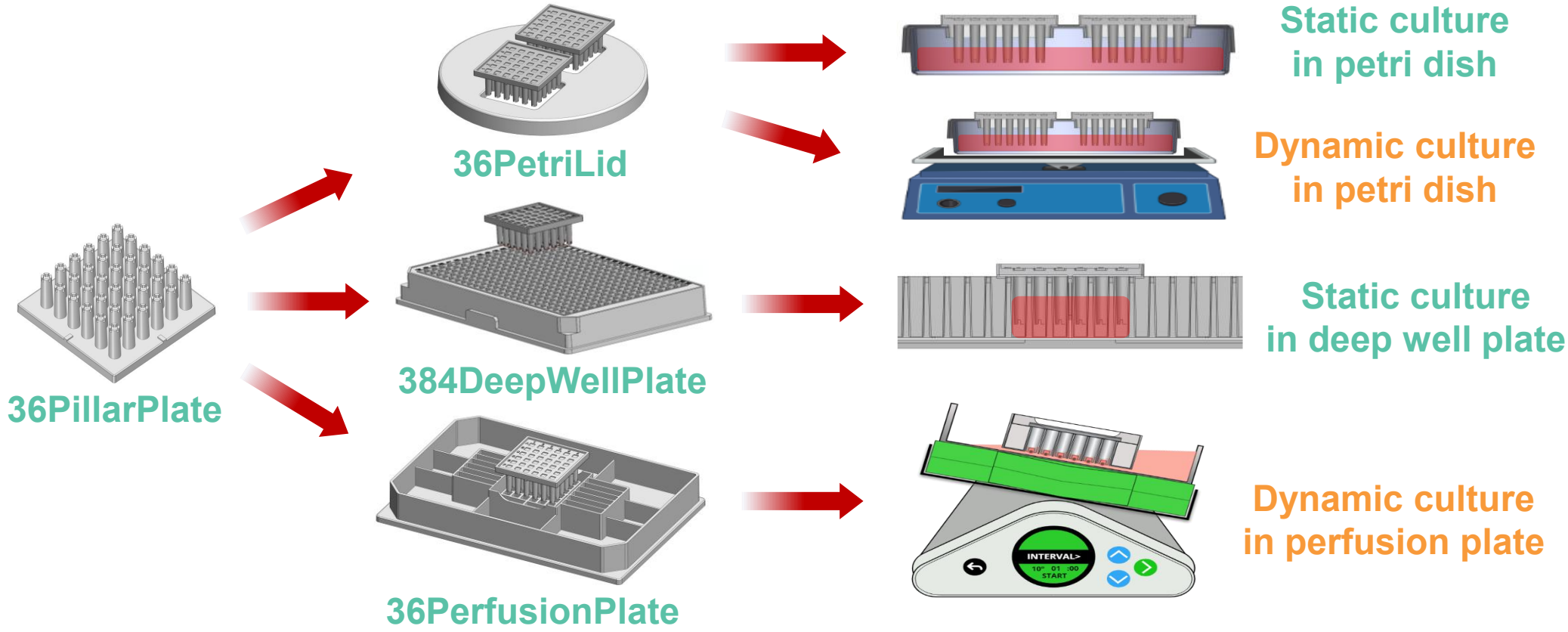


9000 cells/well



Robust spheroid transfer from the ultralow attachment (ULA) 384-well plate to the 36PillarPlate. Scale bars: 500 μm. DOI: 10.1088/1758-5090/ad1b1e

User-Friendly 3D Cell Culture on Pillar Plate



Following cell encapsulation in hydrogels, the pillar plate can be seamlessly integrated with a petri dish, deep well plate, or perfusion plate, enabling both static and dynamic cultures of 3D cells and organoids.

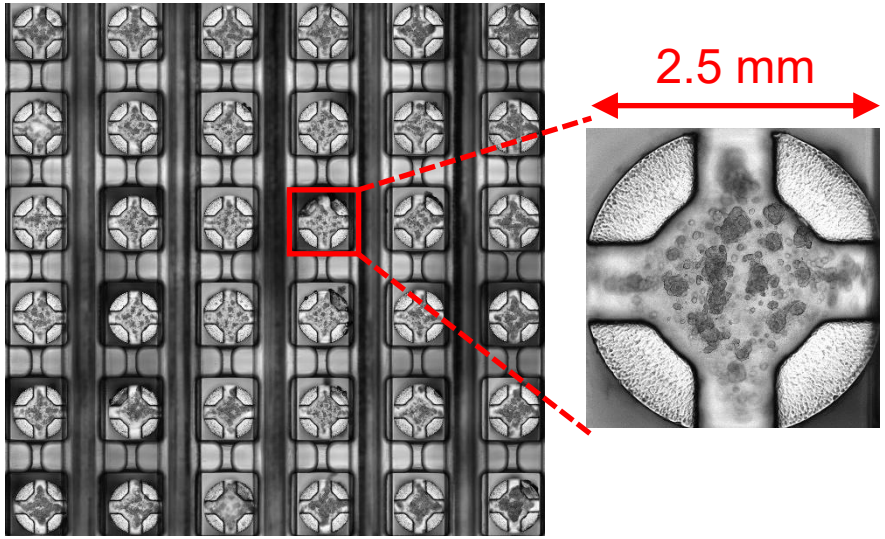
https://youtu.be/buXE65N9kAs

When to Use Dynamic 3D Cell Culture

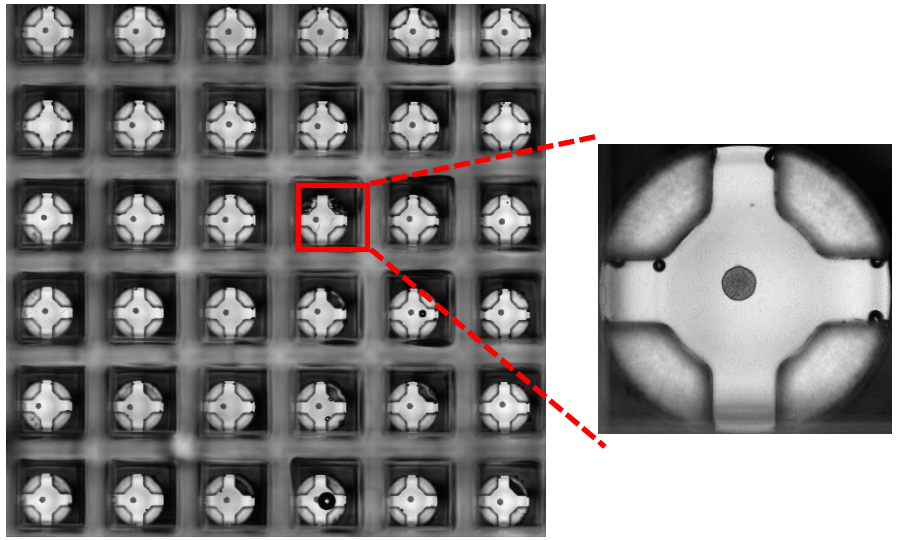
Dynamic 3D cell culture is particularly advantageous under the following conditions:

- **To suppress necrotic core formation** in spheroids and organoids by enhancing mass transport of oxygen and nutrients
- **To promote higher organoid maturity** through improved nutrient delivery and waste removal
- **To evaluate low concentrations of compounds** for effects on cell differentiation and cytotoxicity under physiologically relevant conditions
- **To enhance infection efficiency** of 3D cultures using recombinant viruses *via* improved penetration and distribution
- **To study organoid-organoid communication**, enabling interaction between spatially separated, but fluidically connected organoids
- **To investigate organoid-immune cell interactions** in a dynamic and more physiologically relevant microenvironment

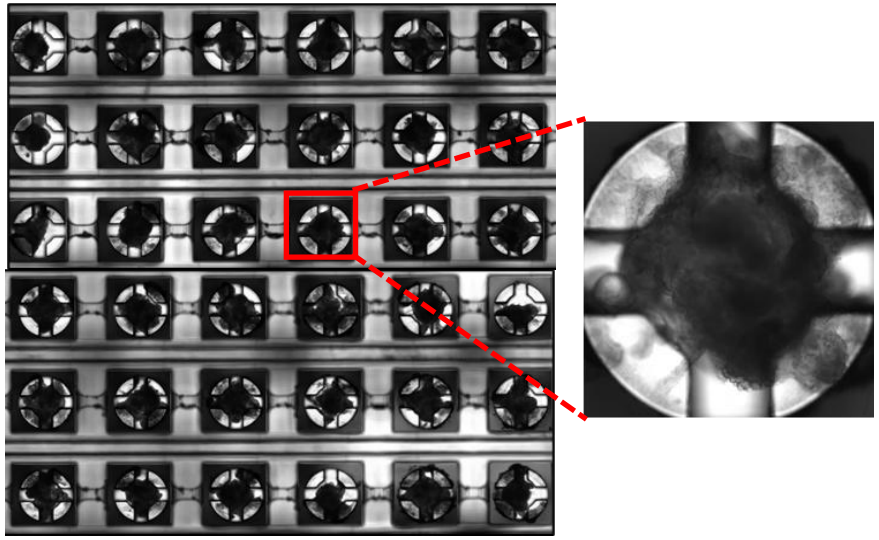
Uniformity of 3D Cell Culture in Pillar/Perfusion Plate



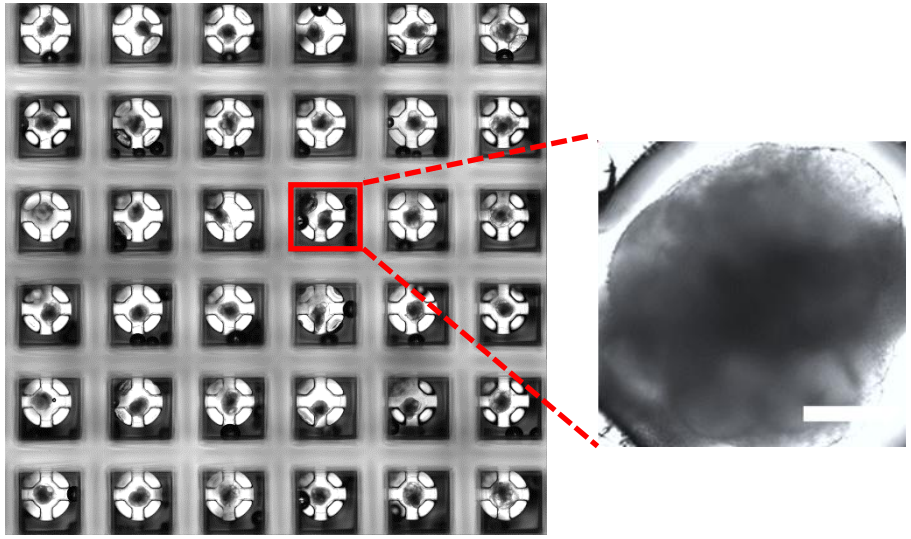
3D-cultured cancer cells



Liver cancer spheroids

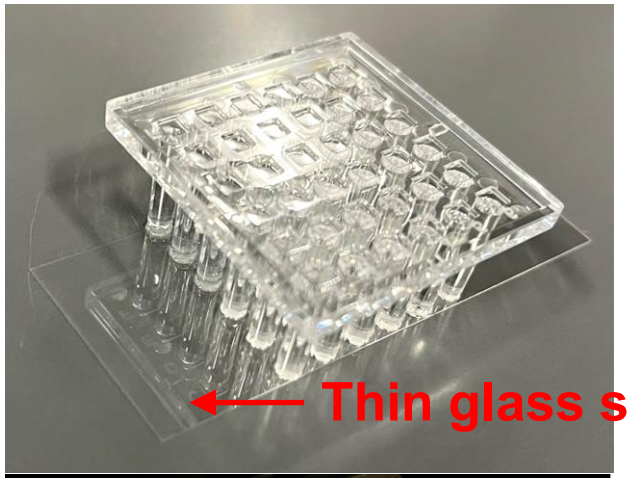


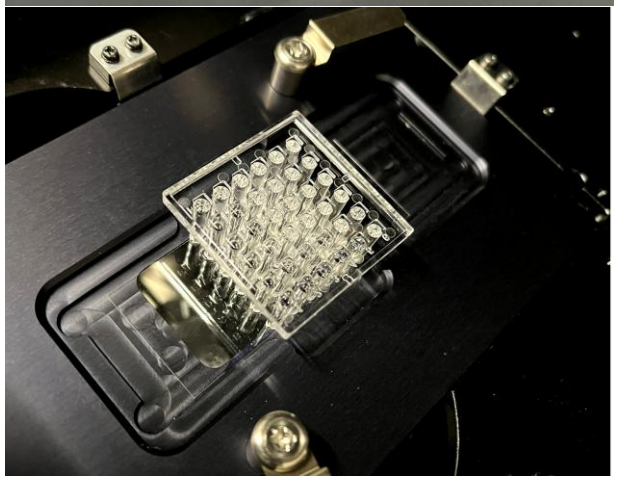
Human liver organoids



Human brain organoids

In Situ 3D Cell Staining and Analysis on Pillar Plate

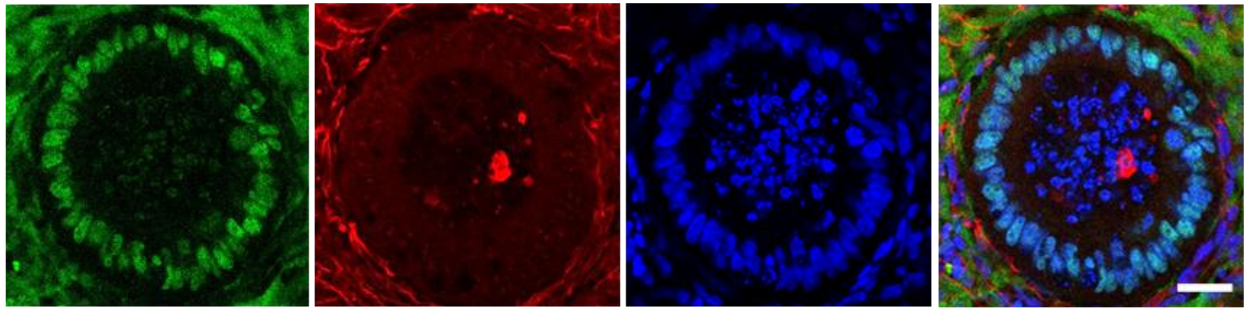




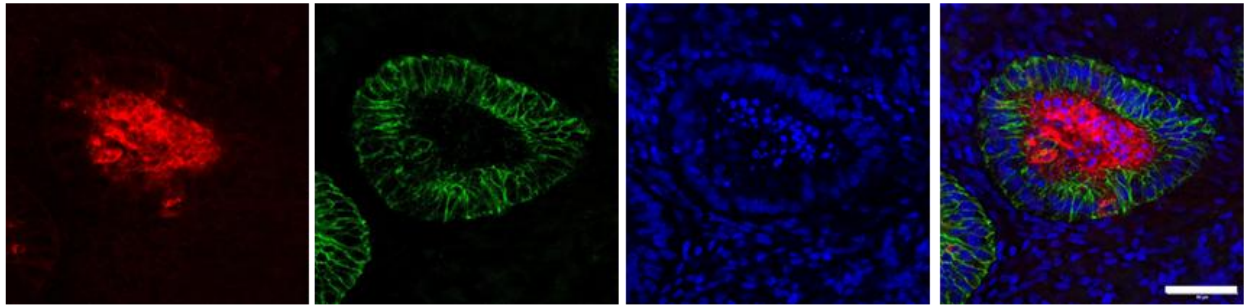
The thin microscopic glass slide attached to the pillar plate with organoids for image acquisition. DOI: 10.1007/7651_2024_603

Human liver organoids

HNF4a/VM/DAPI/Merged

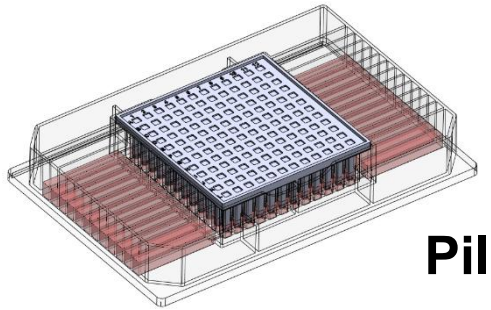


ALB/E-cad/DAPI/Merged

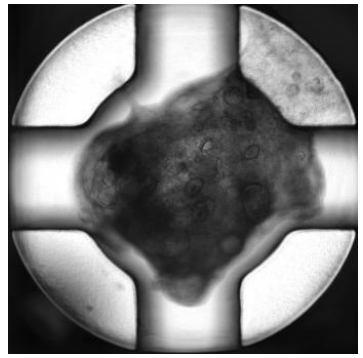


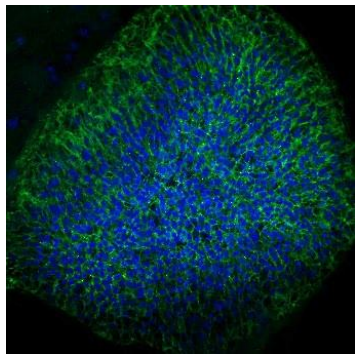
Whole mount immunofluorescence staining of day-25 human liver organoids (HLOs) generated on the pillar plate. Scale bars: 50 μm. DOI: 10.1039/D4LC00149D

Compatibility with Common Lab Equipment



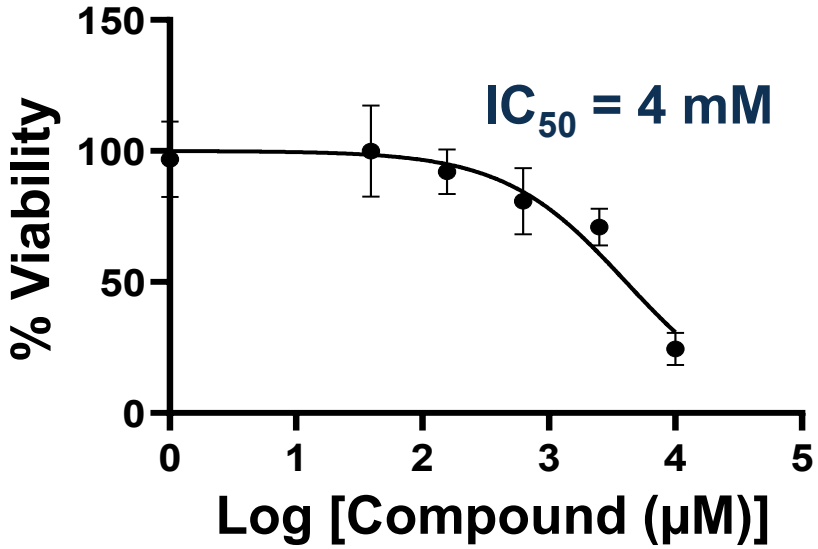
Pillar/perfusion plate





Well plate reader

Fluorescence microscope



The pillar/perfusion plate is fully compatible with standard laboratory equipment for organoid analysis, eliminating the need for customers to invest in additional instrumentation.

Resources from Company Website

Explore resources on our website (3dbpl.com):

- General protocols for experimental workflows
- Application notes demonstrating use cases
- Laboratory training materials for onboarding
- Instructional and demonstration video clips
- Blog posts highlighting insights and updates
- Posters and presentations from conferences
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4099 McEwen Road, Suite 450, Dallas, TX 75244
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