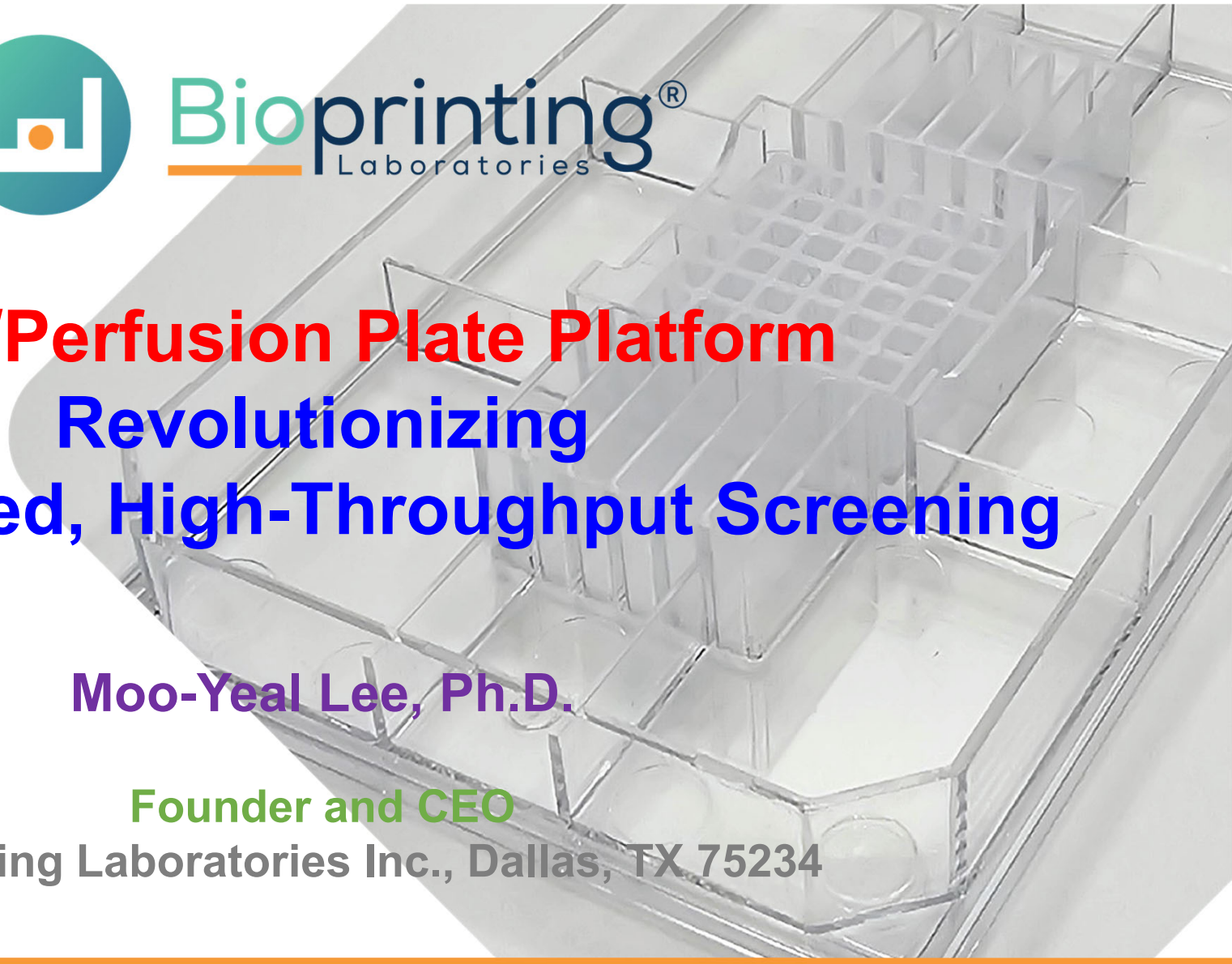
www.3dbpl.com

Bioprinting® Laboratories

# Pillar/Perfusion Plate Platform Revolutionizing 3D Cell-Based, High-Throughput Screening




**Moo-Yeal Lee, Ph.D.**

Founder and CEO

Bioprinting Laboratories Inc., Dallas, TX 75234


*Innovation in Organoid Culture and Disease Modeling*

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## 📌 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.

*Innovation in Organoid Culture and Disease Modeling*

# Why Choose Our Technology?

- ✓ **10-100x assay miniaturization** → Significant cost reduction in drug screening
- ✓ Extremely fast and simple cell/spheroid loading by microarray 3D bioprinting and manual stamping → **HTS friendly**
- ✓ **Enhanced organoid maturity** by dynamic cell culture → More physiologically relevant data
- ✓ Compatible with standard microtiter well plate readers and fluorescence microscopes → **Seamless lab integration**
- ✓ **User-friendly design** → Pump-free, dynamic cell culture without expensive equipment necessary

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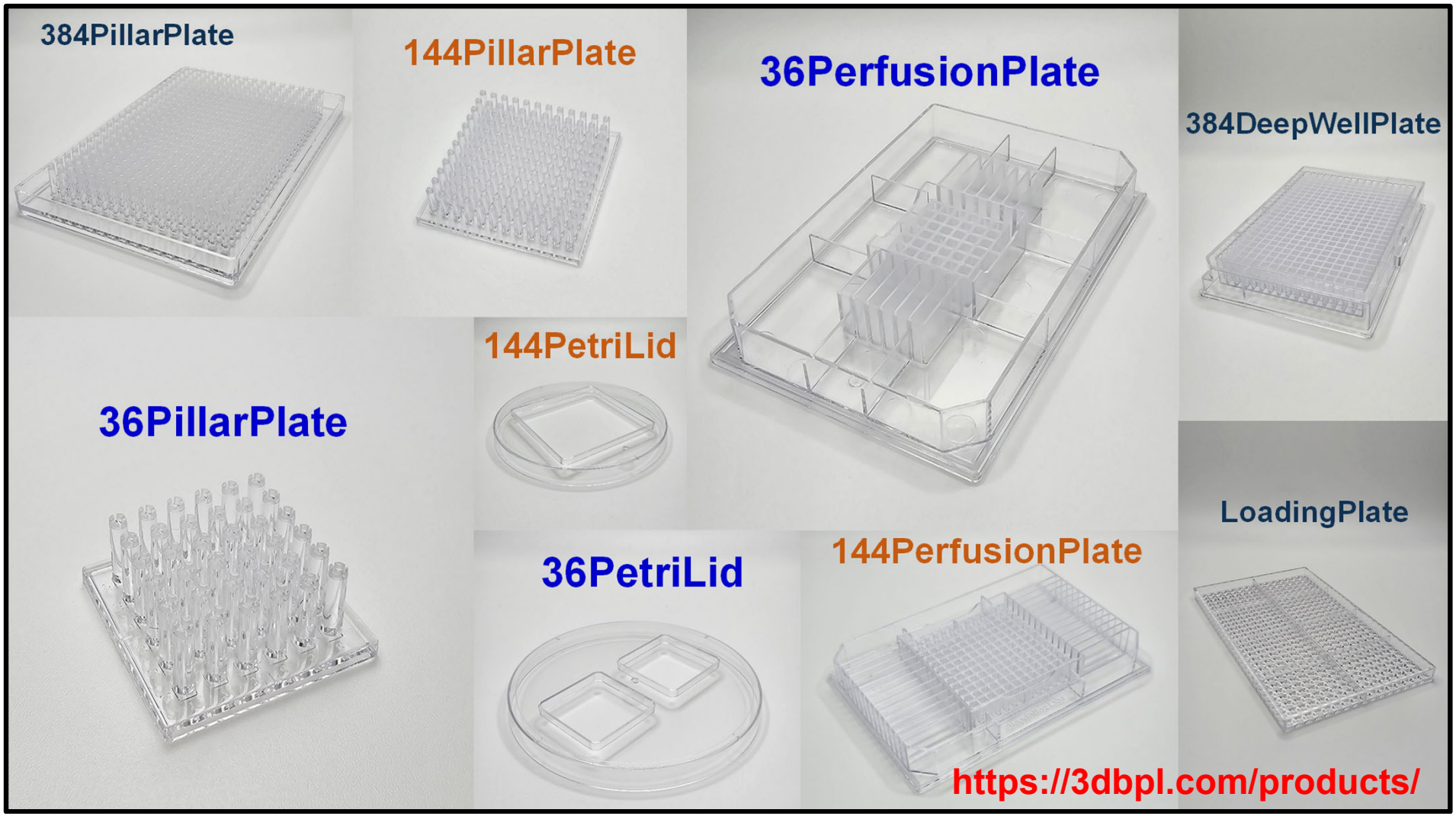
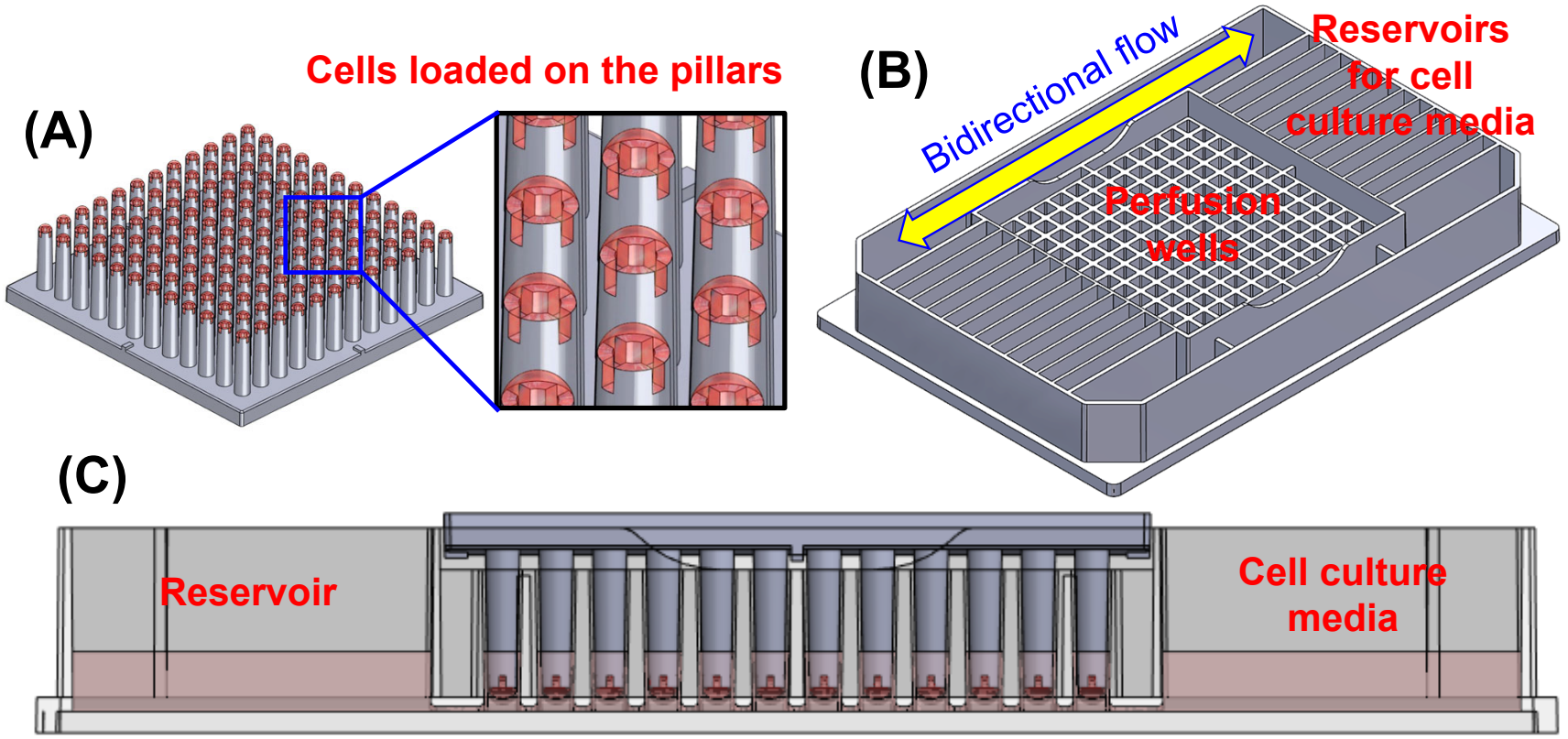
# Our Pillar/Perfusion Plates and Their Accessories for Static and Dynamic Cultures of 3D Cells and Organoids

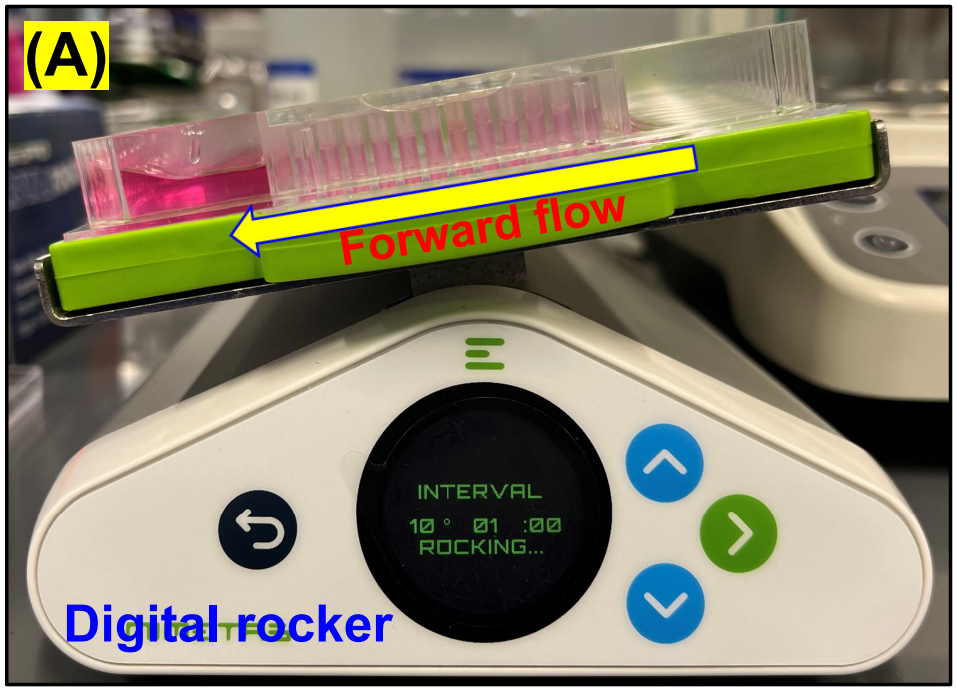


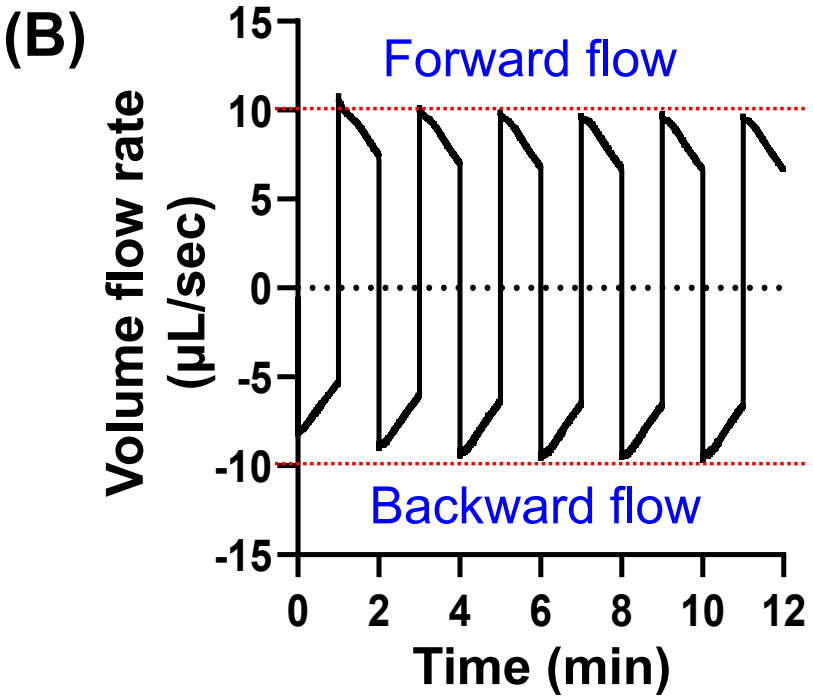
# Pillar/Perfusion Plate for Dynamic Organoid Culture




**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.

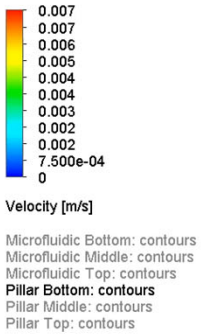
# Pillar/Perfusion Plate for Dynamic Organoid Culture

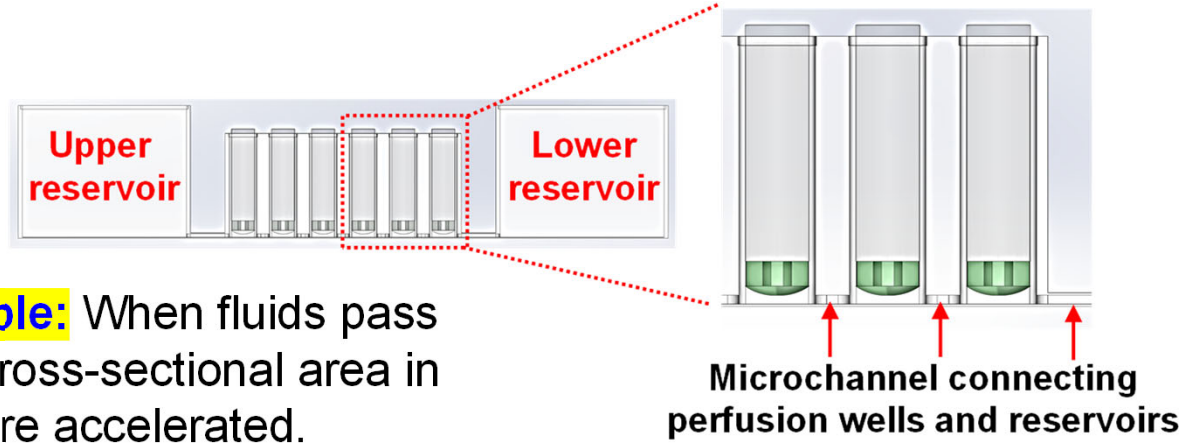


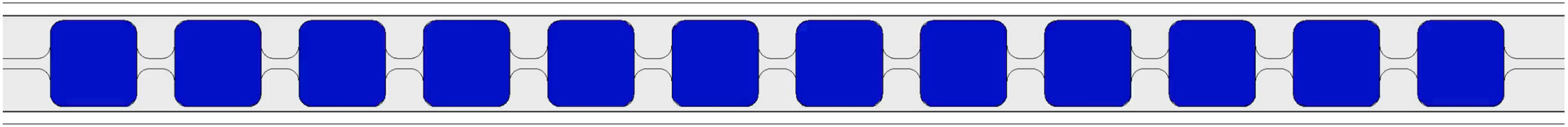

**Our 144PillarPlate and 144PerfusionPlate platform. (A)** The injection-molded pillar/perfusion plate on a digital rocker for dynamic organoid culture. **(B)** Flow rates in the perfusion plate with 1,500 µL culture media at 10° tilting angle and 1 minute frequency of tilting angle change. SolidWorks 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.

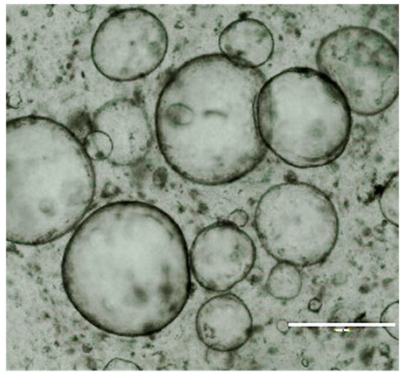


Velocity profiles underneath the pillars in the 144PerfusionPlate over time simulated with SolidWorks with 1500 µL of water at 10° tilting angle, 1 min frequency, and total 4 min running time. The unique design, where pillars are sandwiched into perfusion wells, facilitates rapid mixing of cell culture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

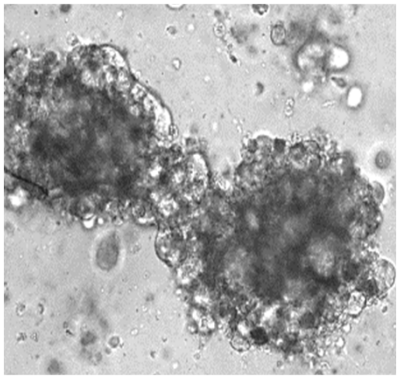
https://youtu.be/irLQIB6Ljnk

# Human Organoids Created in the Pillar/Perfusion Plate

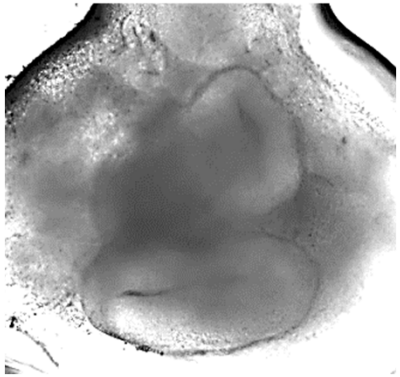
Liver



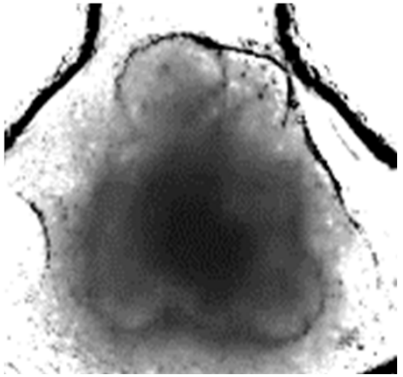
Liver tumor



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)

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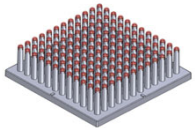
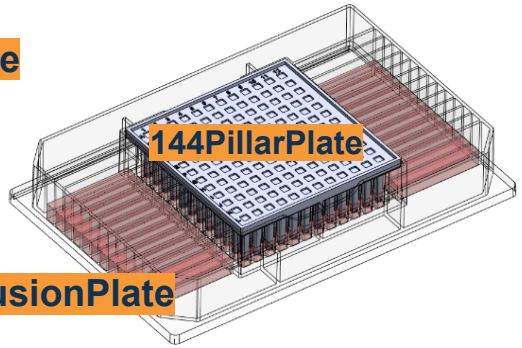
# Unique Features of Pillar/Perfusion Plate Platform for Human Organoid Culture

*Innovation in Organoid Culture and Disease Modeling*

# Cell Culture Platforms for Human Organoids

## 144PillarPlate

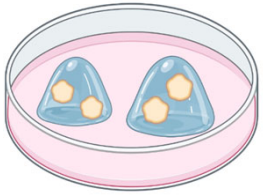





## Benefits:

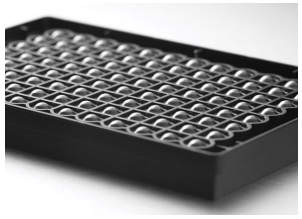
- ✓ Dynamic culture for enhanced diffusion and maturity of organoids
- ✓ Long-term organoid culture with convenient hydrogel embedding
- ✓ High throughput and reproducibility
- ✓ Assay-ready plates with organoids

## 6/24-well plates with Matrigel domes



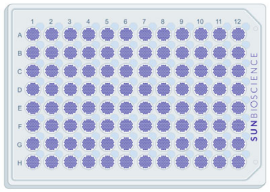
- ✓ Low throughput
- ✓ Poor reproducibility
- ✓ User unfriendliness

## ULA 96-well plate



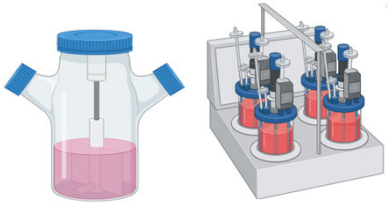
- ✓ Static organoid culture
- ✓ Scaffold-free culture
- ✓ Necrotic core

## Gri3D®96 well plate



- ✓ Static culture for small organoids
- ✓ Scaffold-free culture

## Spinner flasks Mini-bioreactors

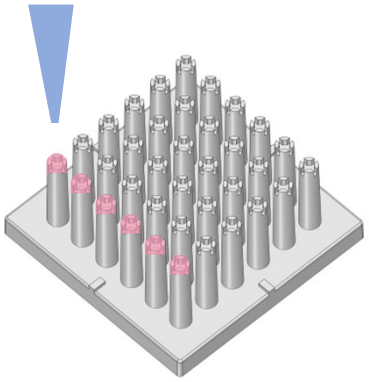


- ✓ Large-scale dynamic organoid culture
- ✓ Non-uniform organoid size
- ✓ Difficult to transfer organoids to well plates

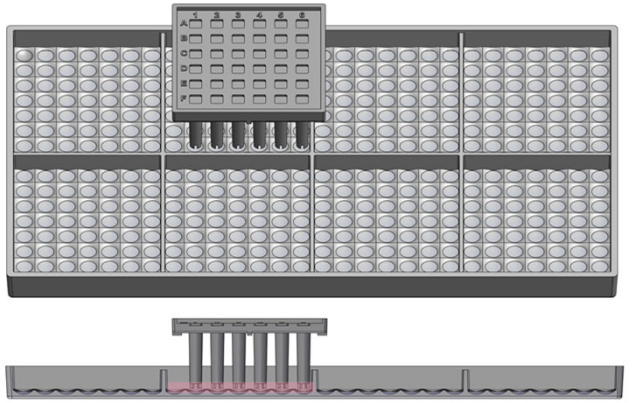
## 48-well PerfusionPal plate

- ✓ Expensive set-up for dynamic culture
- ✓ Low throughput

# Rapid and Robust Cell Loading on the Pillar Plate

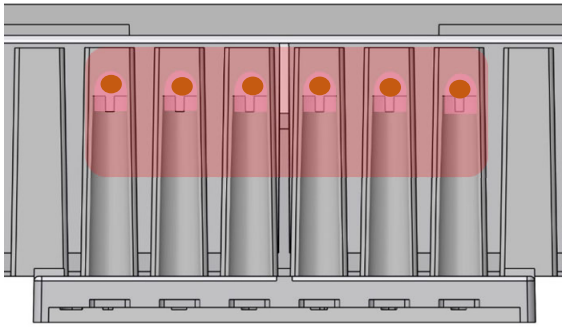


Manual dispensing
with 1 mL pipette tip



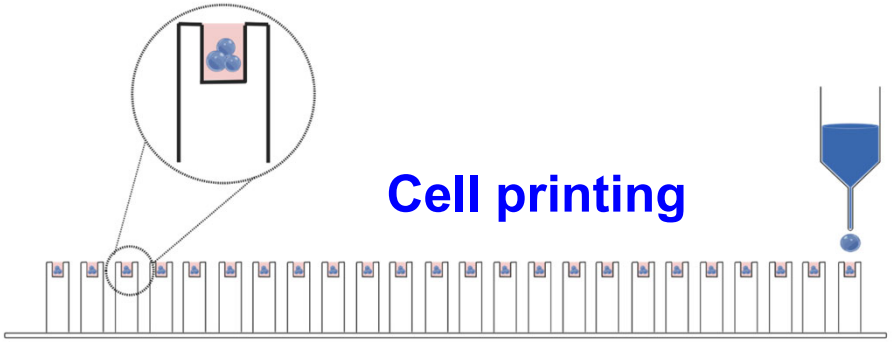
Pillar stamping

ULA 384-well plate



Spheroid
transferring

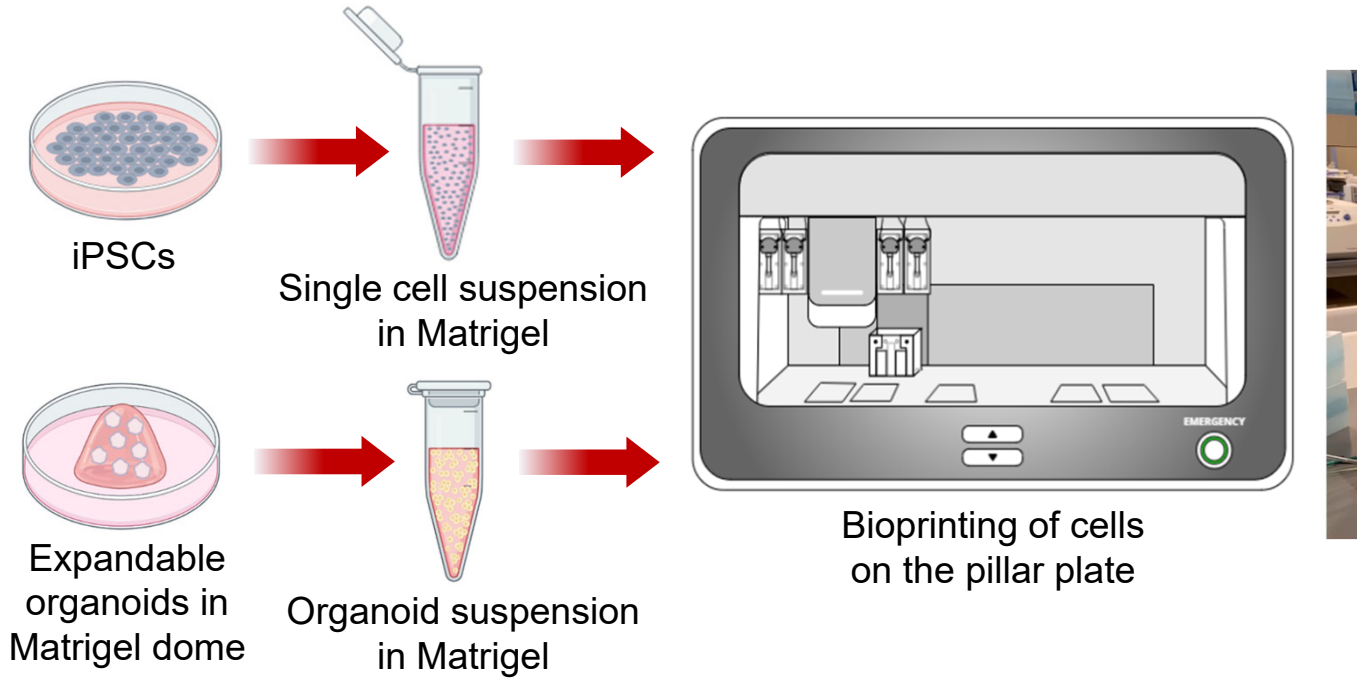
Pillar plate

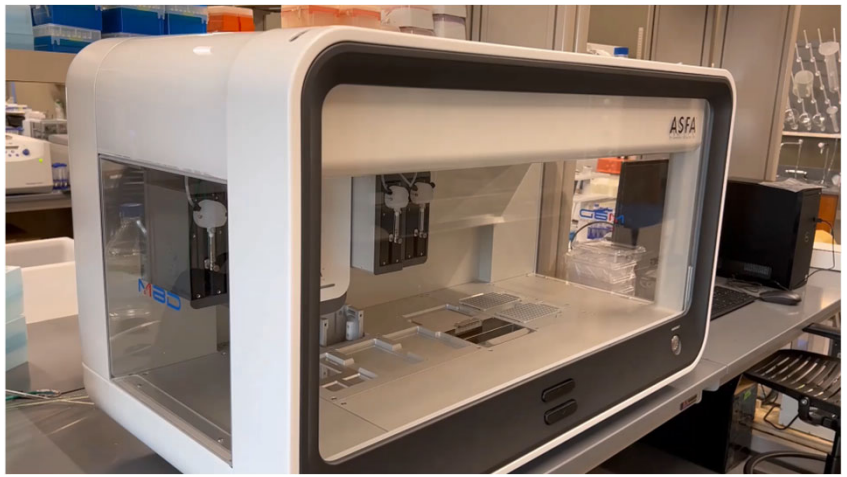


Cell printing

https://youtu.be/EaDw4xoNuel

# Rapid and Robust Cell Loading *via* Microarray 3D Bioprinting

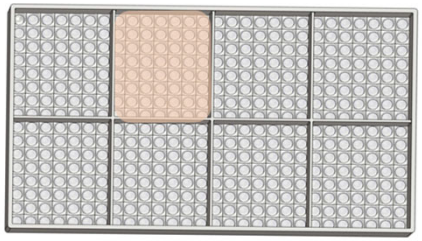


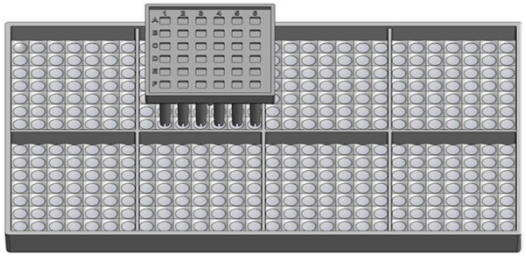

Bioprinting of single cells and spheroids suspended in hydrogels on the pillar plate using a 3D bioprinter. The typical coefficient of variation (CV) for cell printing ranges from 10% to 20%, demonstrating high reproducibility in cell loading *via* 3D bioprinting.

**https://3dbpl.com/resources/**

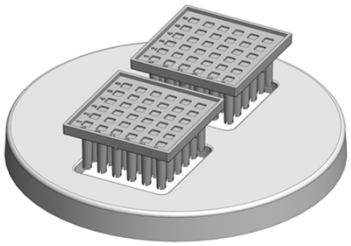
# Rapid and Robust Cell Loading *via* Manual Stamping



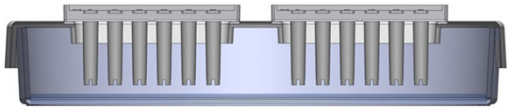
Dispense cell-Matrigel mixture
in the LoadingPlate



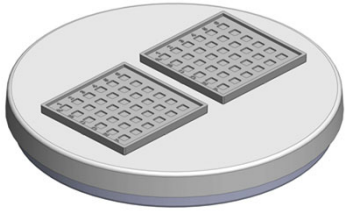
Stamp the 36PillarPlate
to load cells on pillars



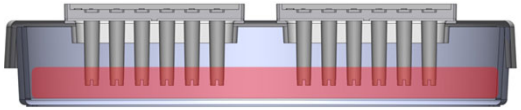
Insert the pillar plate
in the 36PetriLid



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



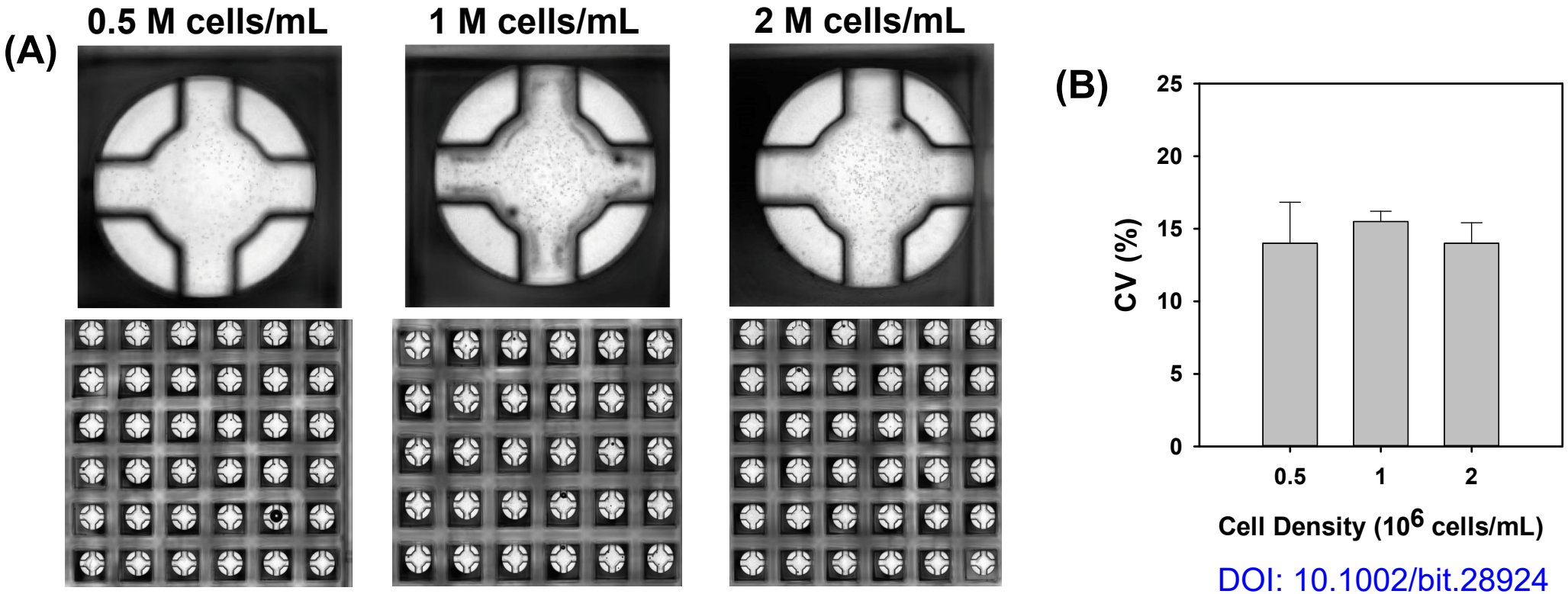
Separate the 36PetriLid
with the pillar plate



Static and dynamic 3D cell culture
in a petri dish (90 mm x 15 mm)

Static and dynamic organoid cultures on the pillar plate in a petri dish *via* manual stamping.

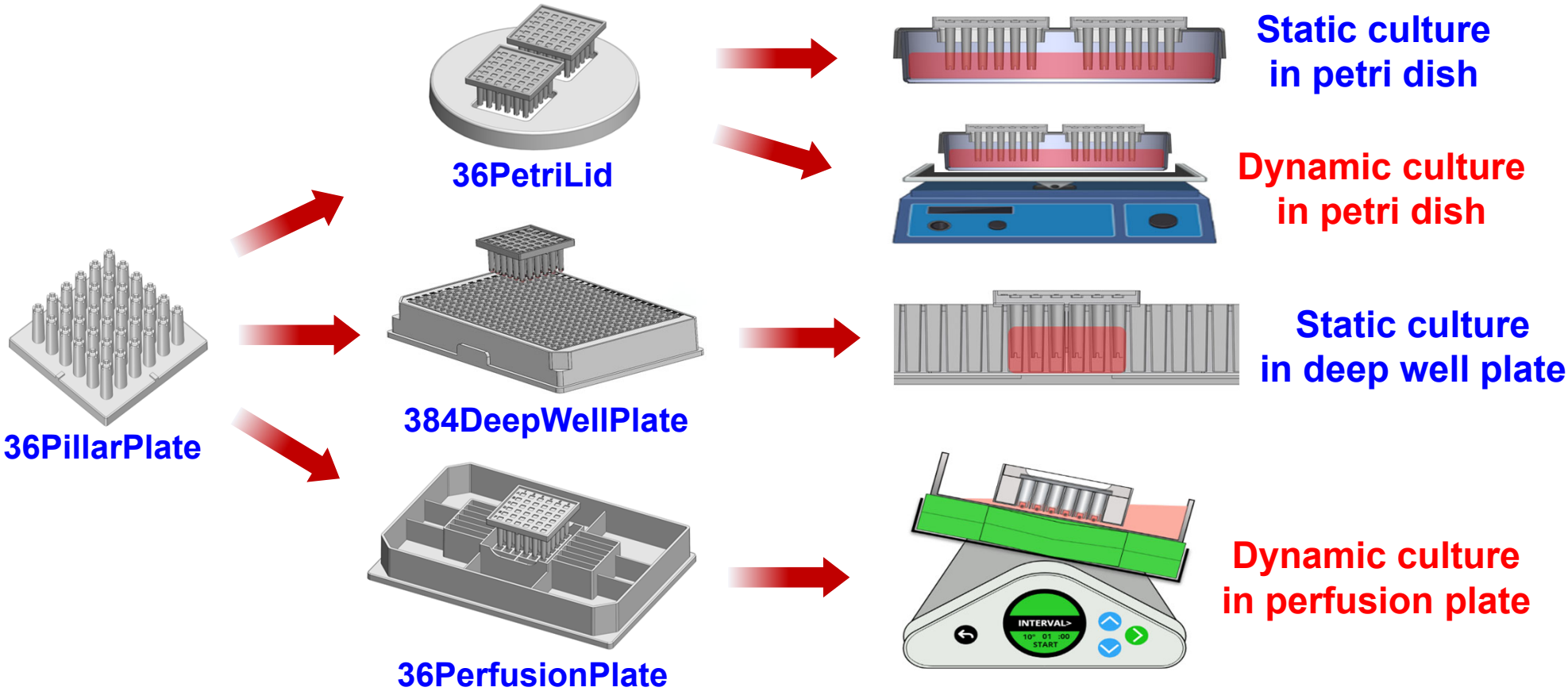
# Reproducibility of Single Cell Loading with the Loading Plate




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.

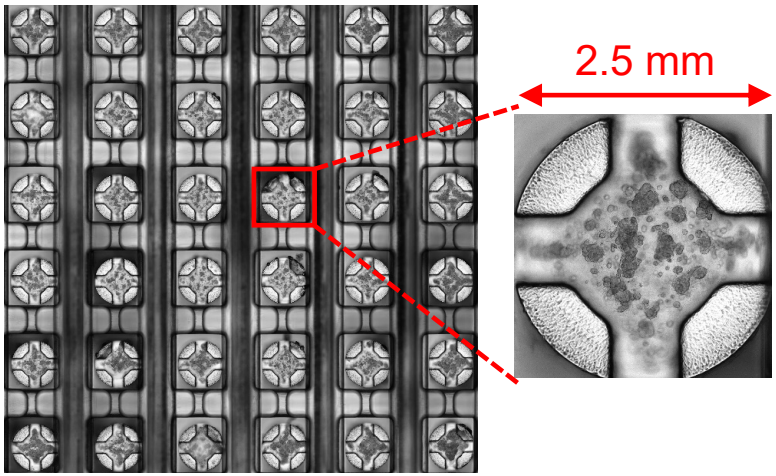
# User-Friendly Organoid Culture on the Pillar Plate



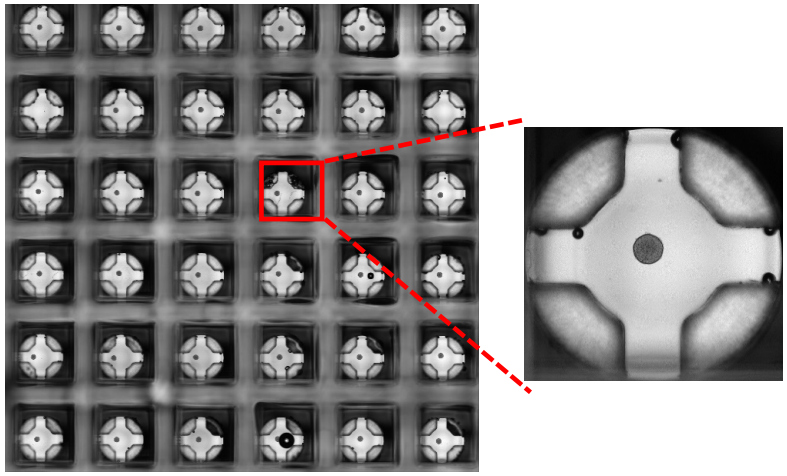

Following cell encapsulation in hydrogels, the pillar plate can be seamlessly integrated with a petri dish, deep well plate, or perfusion plate, enabling both static and dynamic cultures of 3D cells and organoids.

https://youtu.be/buXE65N9kAs

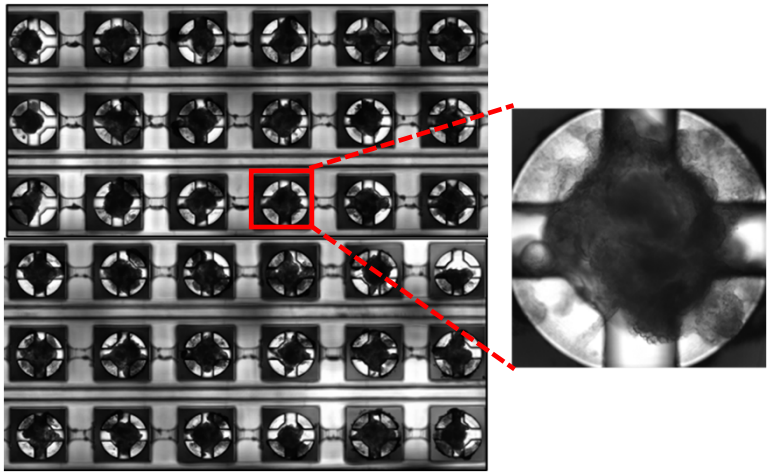
# Uniformity of Organoids in the Pillar/Perfusion Plate



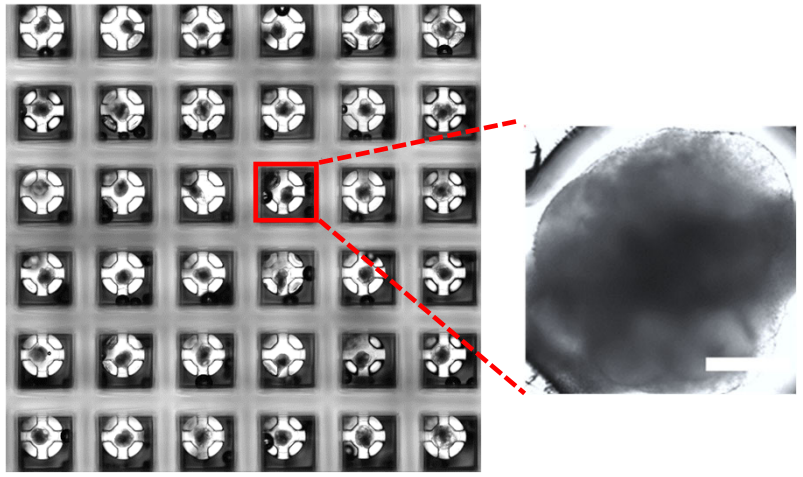

3D-cultured cancer cells



Liver cancer spheroids

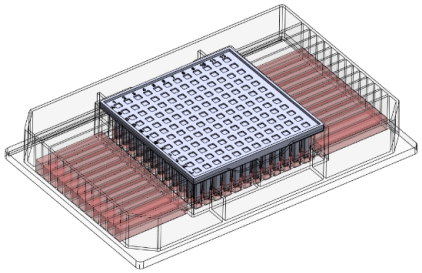


Human liver organoids

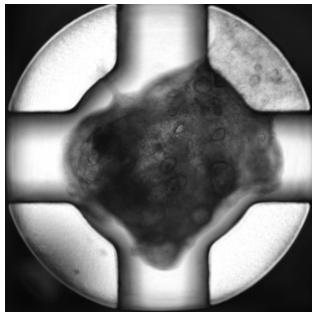


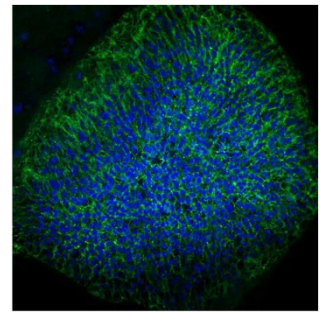
Human brain organoids

# Compatible with Common Lab Equipment for Organoid Analysis



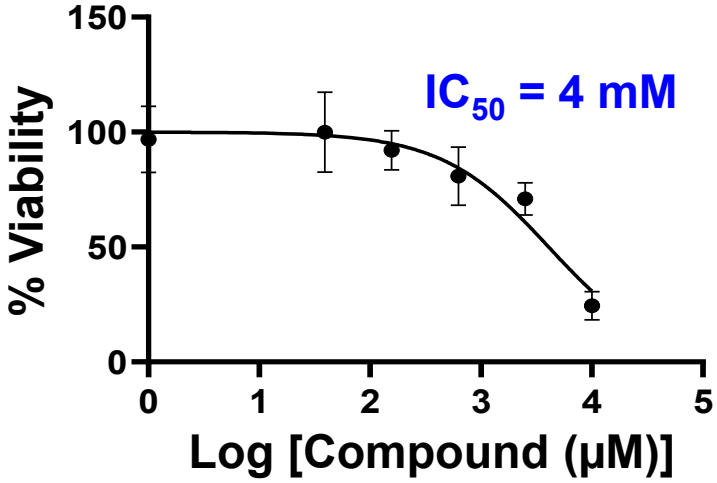
Pillar/perfusion plate





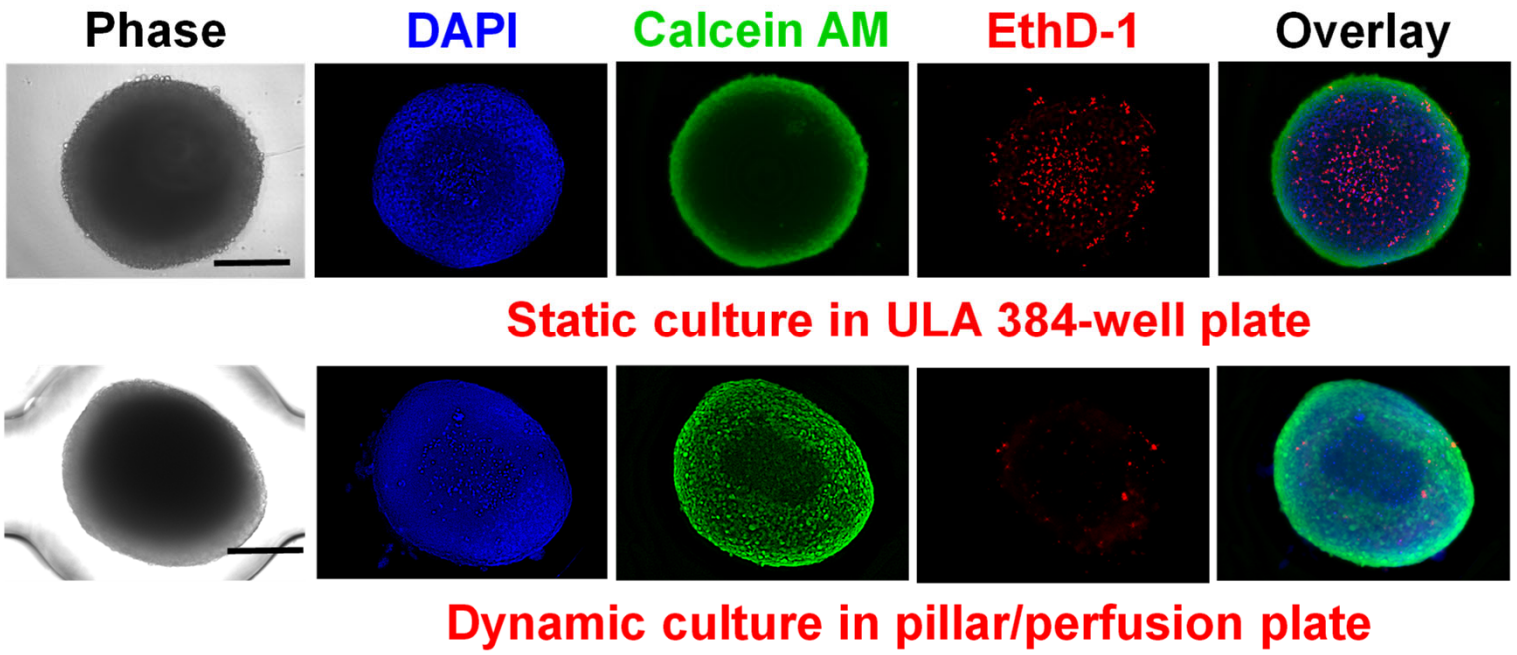

Well plate reader

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.

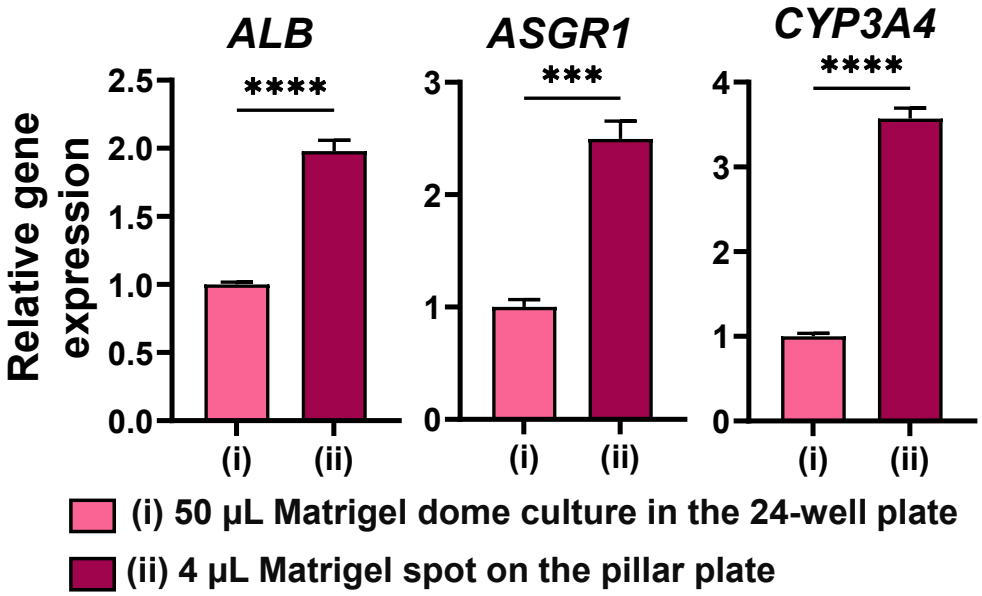
# Reduced Cell Death and Enhanced Organoid Maturity by Rapid Diffusion of Nutrients and Oxygen




Reduced cell death in the core of Hep3B cell spheroids (necrotic core) achieved by dynamic culture in the pillar/perfusion plate. Representative images of Hep3B cell spheroids cultured for 7 days in static and dynamic conditions and stained with calcein AM and ethidium homodimer-1 (EthD-1) to assess cell death in the core. Scale bars: 350 μm. DOI: 10.1021/acsbiomaterials.4c00179

# Reduced Cell Death and Enhanced Organoid Maturity by Rapid Diffusion of Nutrients and Oxygen

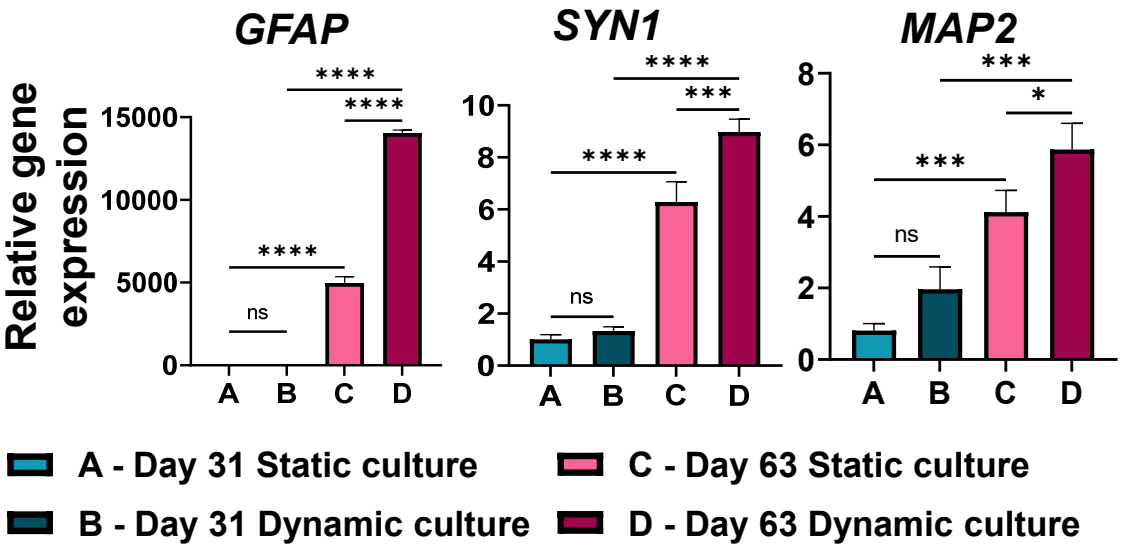
## Human liver organoids




Enhanced maturity of human liver organoids (HLOs) achieved by static culture in the pillar/deep well plates demonstrated by the increased expression of *ALB* albumin marker, *ASGR1* hepatocytes marker, and *CYP3A4* cytochrome P450 3A4 marker as compared to static culture in the 24-well plate.

DOI: 10.1039/D4LC00149D

## Human brain organoids

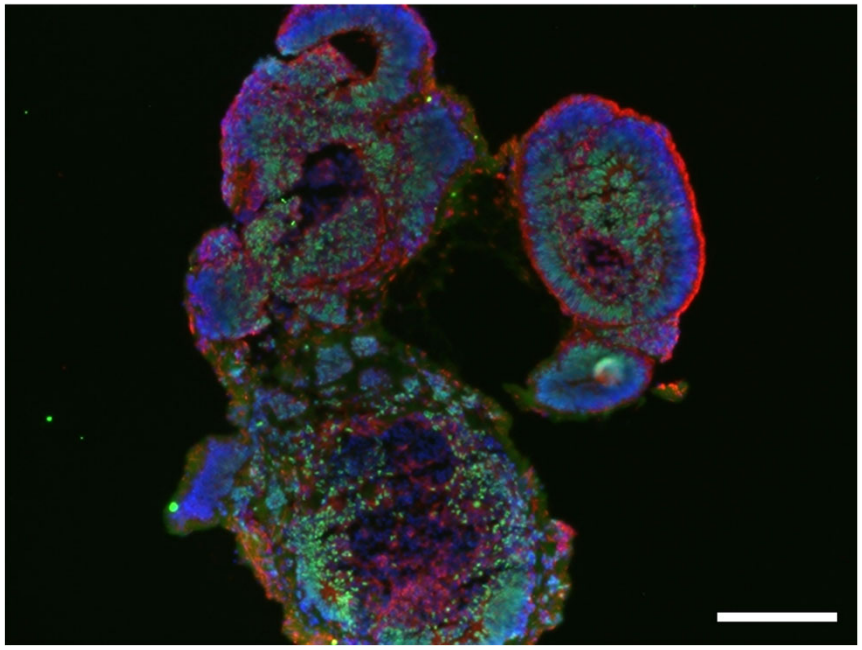



Enhanced maturity of human brain organoids (HBOs) achieved by dynamic culture in the pillar/perfusion plate demonstrated by the increased expression of *GFAP* astrocyte marker, *SYN1* synaptic marker, *MAP2* mature neuronal marker, and *VGLUT1* excitatory neuronal marker as compared to static culture in the pillar/deep well plate.

DOI: 10.1088/1758-5090/ad867e

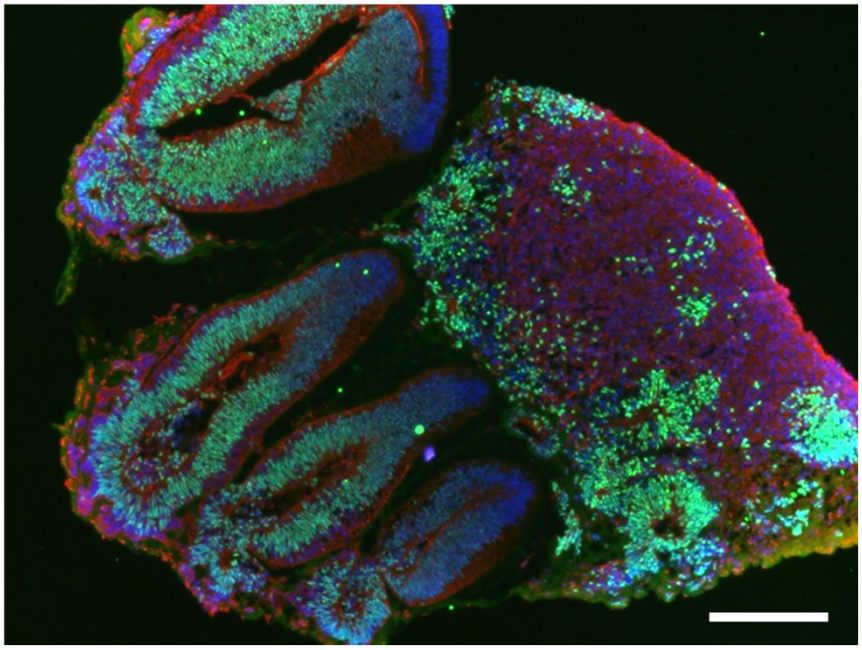
# Higher Maturity of Human Brain Organoids Achieved by Dynamic Culture in the Pillar/Perfusion Plate

TUBB3/SOX2/DAPI



Static culture

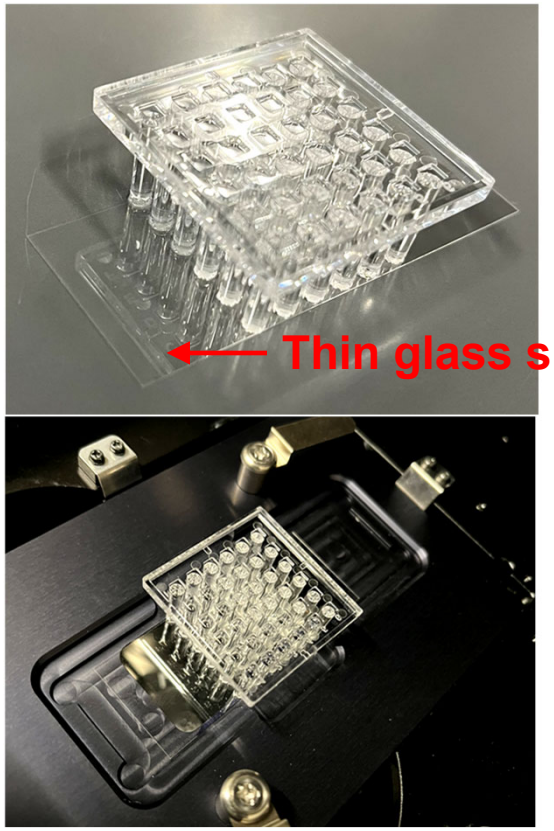
TUBB3/SOX2/DAPI



Dynamic culture

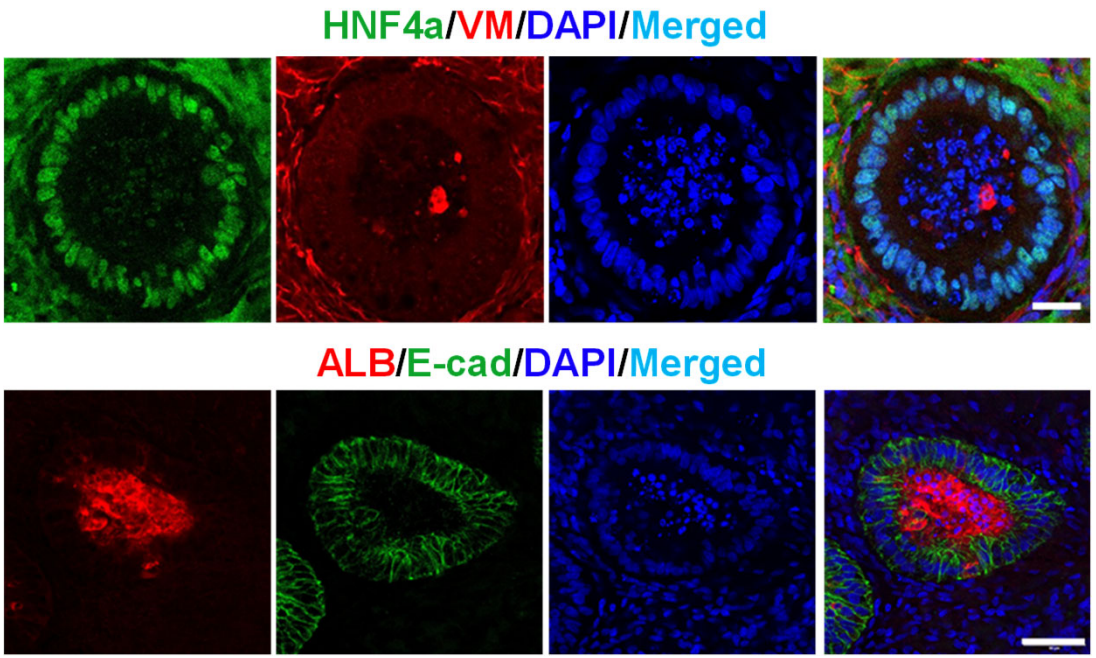
Immunofluorescence staining of cryo-sectioned brain organoids cultured in static and dynamic conditions for 31 days on the pillar plate. Scale bars: 200 μm. The layered structure is notably more pronounced in brain organoids cultured dynamically in the pillar/perfusion plate, highlighting the impact of dynamic culture on organoid development. DOI: 10.1088/1758-5090/ad867e

# *In Situ* Organoid Testing and Whole Mount Imaging




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

## Human liver organoids

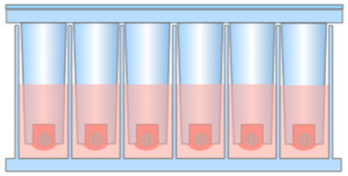


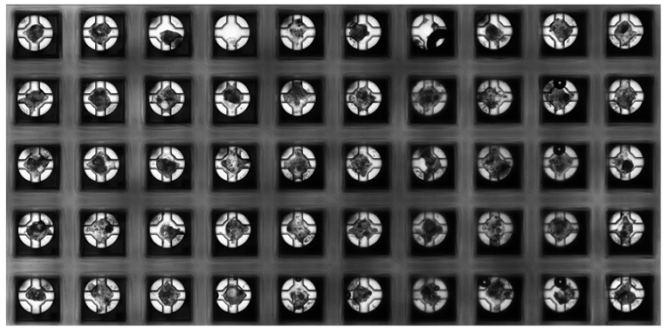

Whole mount immunofluorescence staining of day-25 human liver organoids (HLOs) generated on the pillar plate. Scale bars: 50 μm. DOI: 10.1039/D4LC00149D

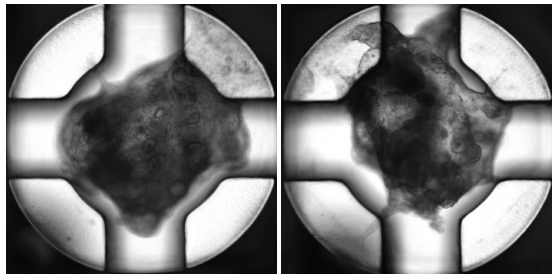
# Scale-up Production with Expandable Human Liver Organoids

## Static Culture

(A)

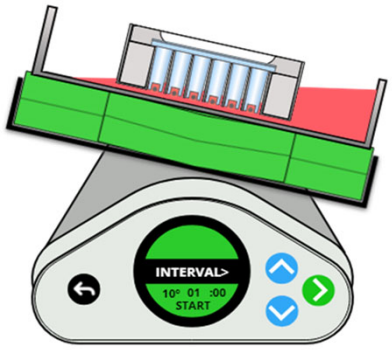


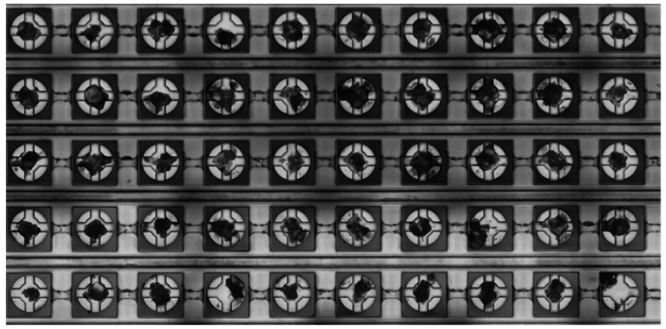


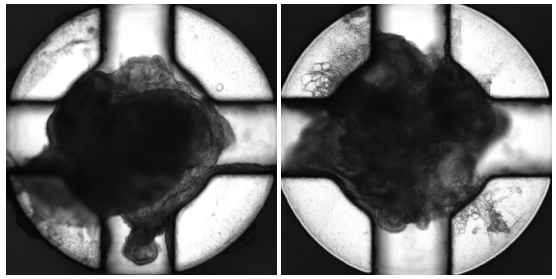


## Dynamic Culture

(B)



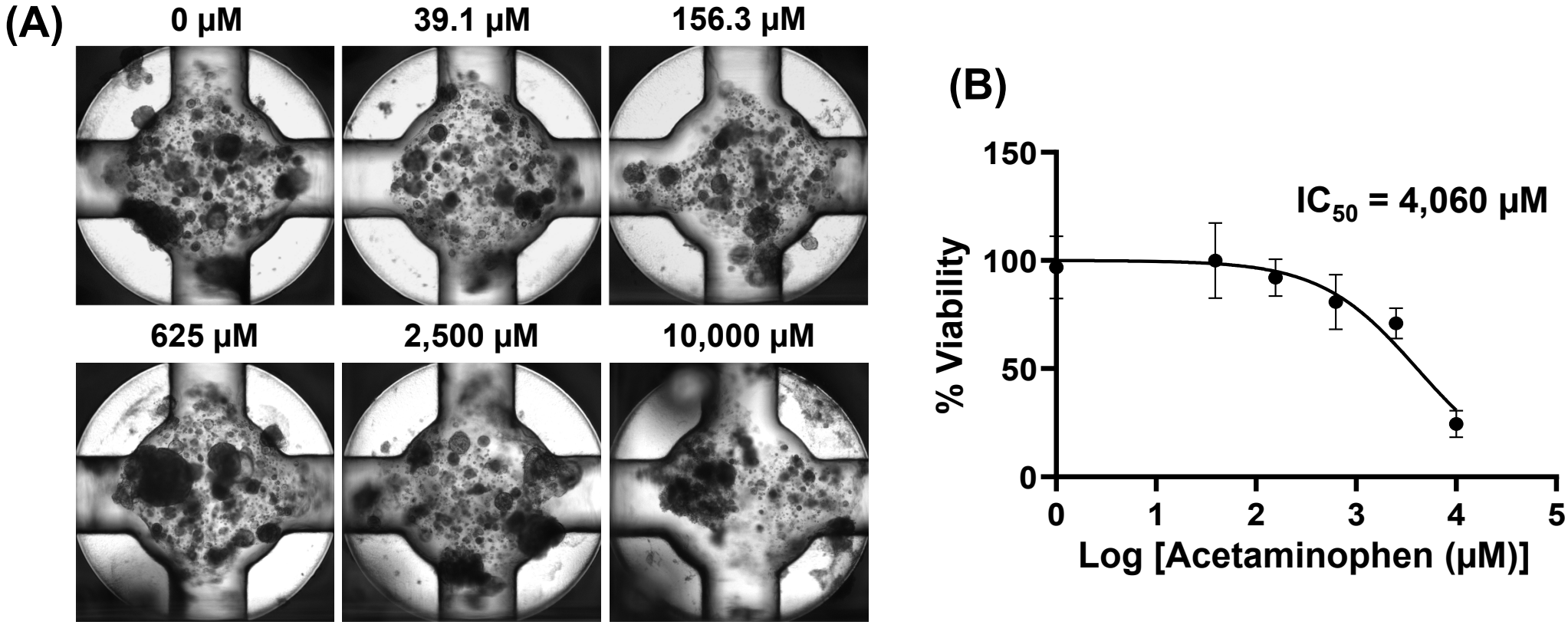





**(A)** Stitched (left) and representative (right) images of day-20 human liver organoids (HLOs) from expandable HLOs cultured in a static condition using the 144PillarPlate and the 384DeepWellPlate. **(B)** Stitched (left) and representative (right) images of day-20 HLOs cultured in a dynamic condition using the 144PillarPlate and the 144PerfusionPlate.

DOI: doi.org/10.1101/2024.03.25.586638

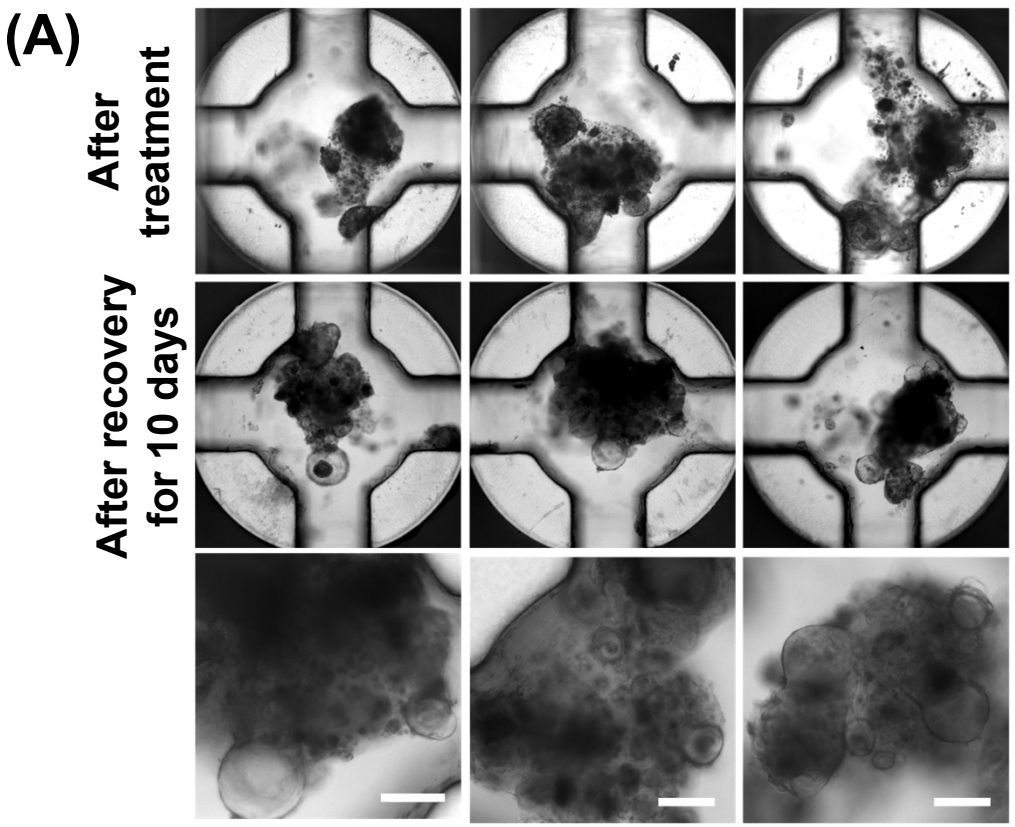
# Assessment of Hepatotoxicity with Human Liver Organoids

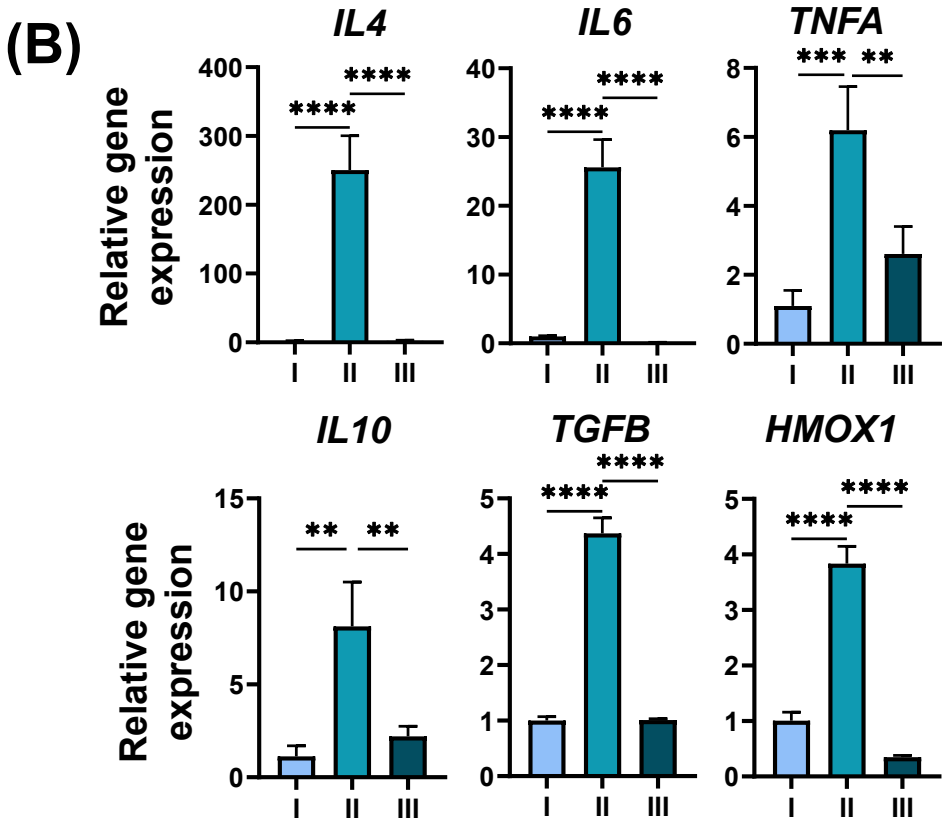



Assessment of hepatotoxicity with acetaminophen with day-20 human liver organoids (HLOs) in the pillar/perfusion plate. **(A)** Representative images of HLOs after acetaminophen treatment for 3 days at the concentration range of 0 – 10,000 µM and **(B)** its dose response curve.

DOI: doi.org/10.1101/2024.03.25.586638

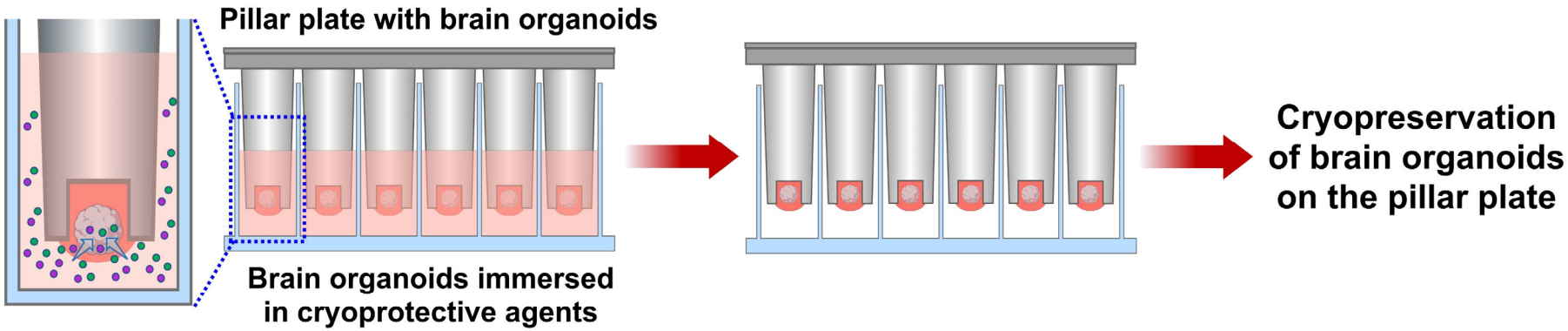
# Assessment of Liver Organoid Regeneration after Treatment



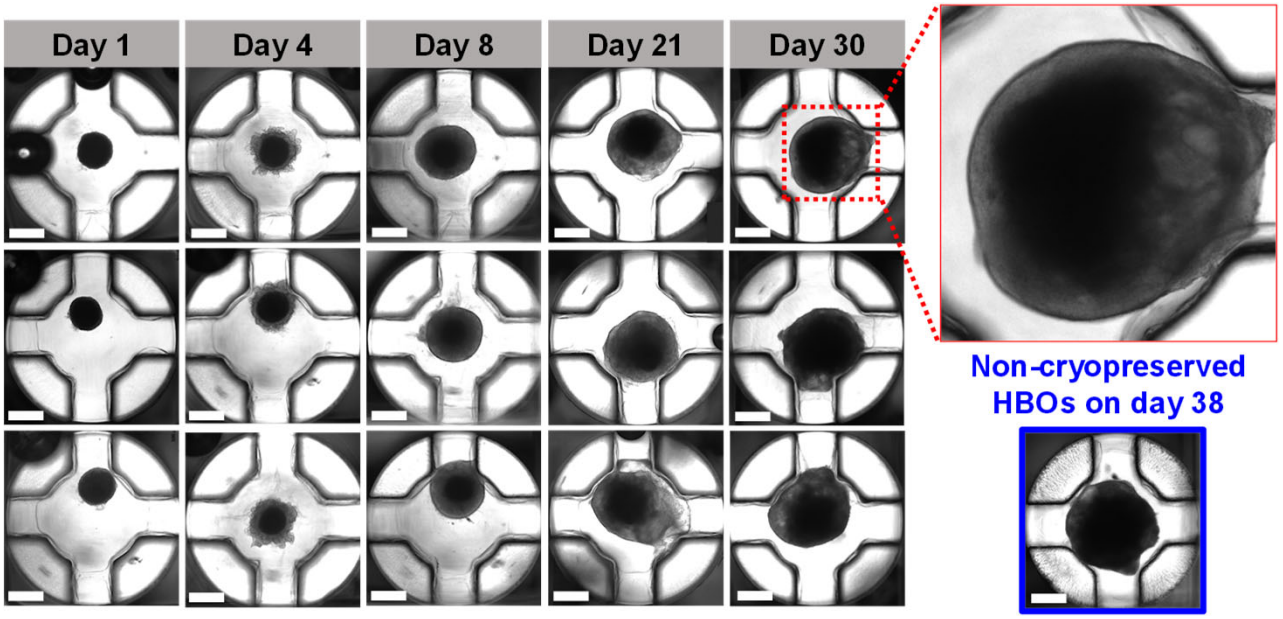




Assessment of liver organoid regeneration after acetaminophen treatment in the pillar/perfusion plate. **(A)** Representative images of HLOs after 3 days of treatment with 5,000 μM acetaminophen (top panel) and after 10 days of organoid regeneration in the expansion medium (middle and bottom panels). Scale bars: 200 μm. **(B)** Changes in inflammatory gene expression in (I) HLOs (control), (II) HLOs after 3 days of acetaminophen treatment, (III) HLOs after 10 days of regeneration. n = 12.

# On-Chip Cryopreservation of Human Organoids



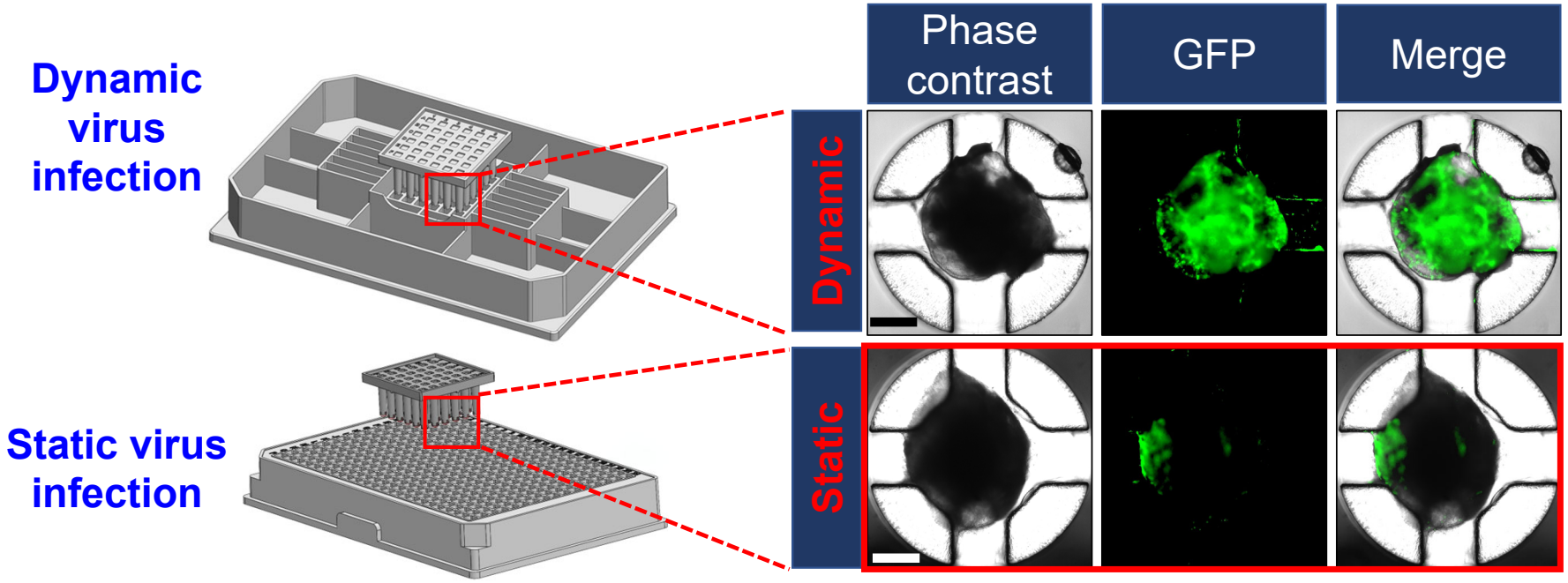

**On-chip cryopreservation of human organoids encapsulated in Matrigel on the pillar plate for high-throughput, organoid-based assays**




The changes in the morphology of neuroectoderms (NEs) by differentiation on the pillar plate for 1, 4, 8, 21, and 30 days after cryopreservation and thawing. The cryopreserved NEs were properly recovered and showed the expansion of neuroepithelium on day 4 after differentiation. Scale bars: 500 μm.

DOI: 10.1021/acsbiomaterials.4c01383

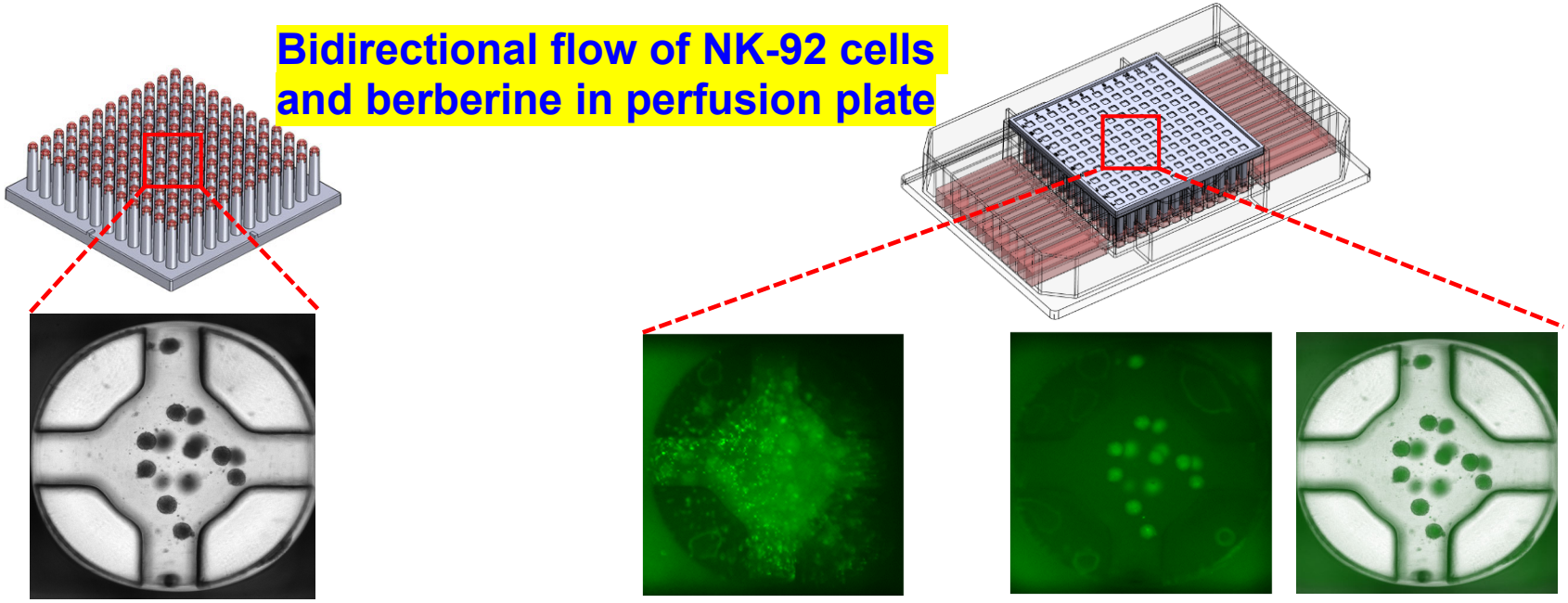
# High Efficiency of Dynamic Virus Infection in Brain Organoids




Day-35 brain organoids infected with AAV9 carrying GFP under dynamic and static conditions in the pillar/perfusion plate. Scale bars: 500 μm. A viral load of 1.74 x $10^8$ genomes was applied per organoid. The pillar/perfusion plate demonstrated high efficiency in organoid virus infection, highlighting its critical role in gene editing for human organoids and disease modeling.

# Dynamic Immune Cell-Mediated Killing of Liver Tumor Cells in the Pillar/Perfusion Plate

**Bidirectional flow of NK-92 cells and berberine in perfusion plate**



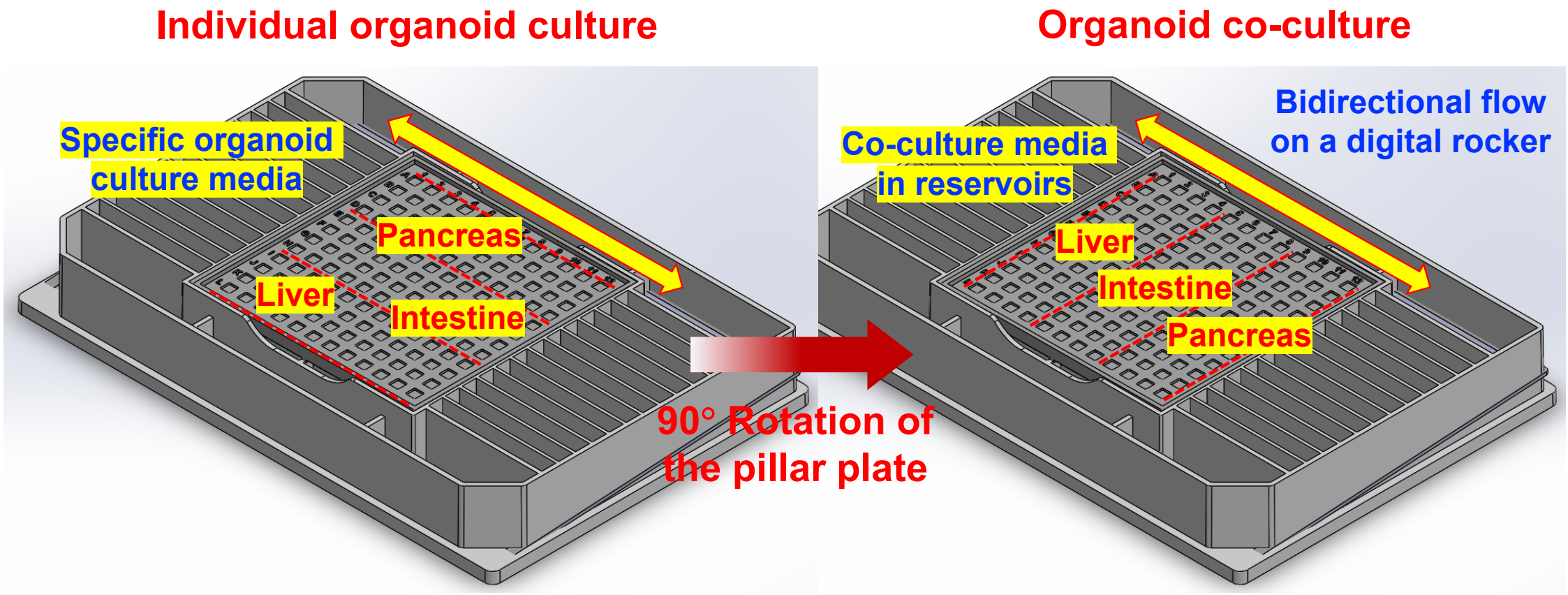
**Hep3B spheroids in Matrigel printed on the pillar plate**

**CellTracker Green-stained NK-92 cells in perfusion wells**

**Infiltration of NK-92 cells into Hep3B spheroids encapsulated in Matrigel**

Berberine-enhanced NK-92 cytotoxicity against Hep3B tumor spheroids under dynamic conditions in the pillar/perfusion plate. A 1:1 ratio of Hep3B and NK-92 cells was treated with 5 μM berberine over 3 days. Immune cell infiltration into liver tumor spheroids, followed by immune cell-mediated cytotoxicity, was observed in the pillar/perfusion plate.

# Multi-Organoid Interactions in the Pillar/Perfusion Plate




Dynamic human organoid culture on the 144PillarPlate and the 144PerfusionPlate and fluidic organoid-organoid interactions for disease modeling and predictive drug screening.

# How You Can Get Involved

- **Pharma & Biotech:** Partner with us for validation studies
- **Investors:** Support our scale-up and commercialization efforts
- **Researchers:** Request demo/sample at **3dbpl.com**

***Innovation in Organoid Culture and Disease Modeling***

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## Problem

Physiologically irrelevant 2D cell culture in drug discovery processes

## Solution

Human organoid culture in pillar/perfusion plates for high-throughput screening (HTS) of compounds

## Technology

Pillar/perfusion plates combining microarray 3D bioprinting and dynamic organoid culture for predictive drug tests

## IP/Regulation

Exclusive, worldwide license for key patents

No federal regulatory approval needed

## Market

$200M organ-on-chip market

5% penetration ($10M) in 5 years by product sales

## What We Need

Recruitment, connection and financial investment