Bioprinting® Laboratories

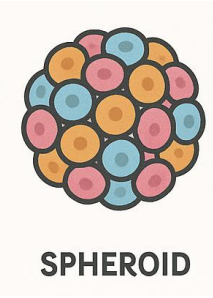
Day 1: Introduction & System Overview

www.3dbpl.com

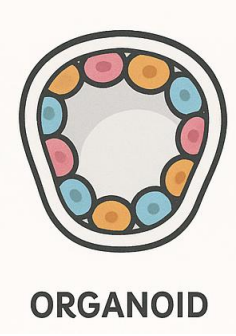
Innovation in Organoid Culture and Disease Modeling

3D Cell Models as New Approach Methodology (NAM)

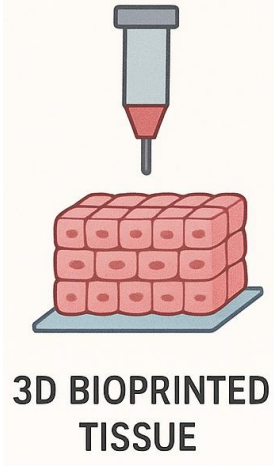
- **Spheroids**
  - **Definition:** Simple cell aggregates formed through self-assembly
  - **Applications:** Tumor modeling, drug screening and toxicity testing, and immunotherapy evaluation



- **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



- **Bioprinted tissue constructs**
  - **Definition:** Layer-by-layer deposition of bioinks (cells + hydrogels) using advanced printing methods, such as inkjet, extrusion, and laser-assisted bioprinting
    - Spatial control over cell distribution and ECM components
    - Ability to design complex, customized 3D constructs
  - **Applications:** Regenerative medicine, tissue engineering, and drug testing



Methods of 3D Cell Structure Formation: Scaffold-Free

- **Hanging droplet plates**
  - Small droplets of cell suspension are placed on an inverted plate.
  - Gravity drives cells to aggregate at the bottom, forming spheroids.

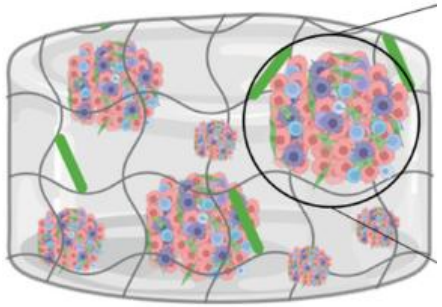
- **Ultralow attachment (ULA) well plates**
  - Plates are coated with non-adhesive polymers (e.g., agarose, poly-HEMA).
  - Prevents cell attachment to the surface, promoting cell-cell interactions and spheroid formation.

- **Spinner flasks / Rotating wall bioreactors**
  - Provide continuous mixing to suspend cells.
  - Prevents sedimentation and enhances formation of spheroids and organoids by dynamic aggregation.

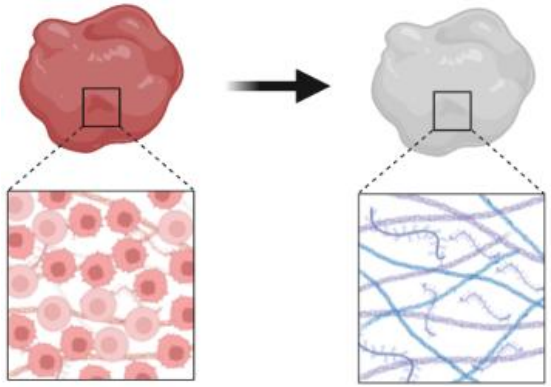
- **Magnetic levitation**
  - Cells are tagged with magnetic nanoparticles.
  - Magnetic fields levitate and assemble them into 3D aggregates.

Methods of 3D Cell Structure Formation: Scaffold-Based

- **Hydrogels (natural or synthetic)**
  - Provide ECM-like environment to support cell adhesion, differentiation, and tissue development.
  - Examples: Collagen, Matrigel, alginate, hyaluronic acid, PEG, fibrin.



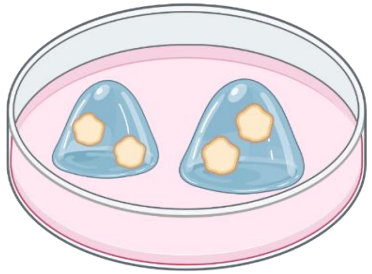
- **Decellularized extracellular matrix (ECM)**
  - Tissues are stripped of cellular components while retaining native ECM structure and bioactive signals.
  - Offers a highly biomimetic environment for cell attachment and organ-specific function.



- **Porous scaffolds**
  - Sponge-like materials (e.g., PLGA, ceramics) with controlled pore sizes.
  - Facilitate tissue ingrowth, vascularization, and nutrient diffusion.

3D Cell Culture Devices

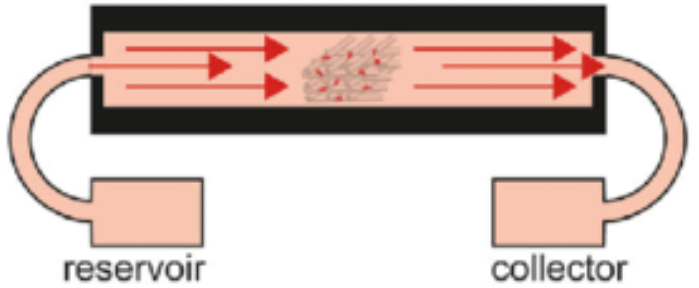
- **Microtiter well plates & Petri dishes**
  - Standard labware commonly adapted for 3D culture.
    - Support static 3D cultures (spheroids, organoids).
    - Can be integrated with dynamic culture platforms (e.g., digital rockers, orbital shakers).
  - **Advantages:** High-throughput compatibility, cost-effective.
  - **Limitations:** Limited nutrient diffusion and oxygen penetration in static setups.



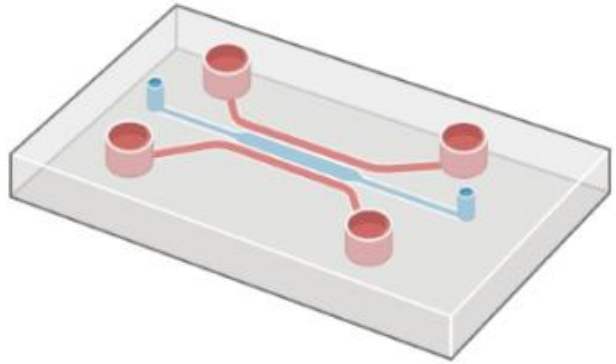
- **Spinner flasks & Rotating wall bioreactors**
  - Bioreactors that maintain constant mixing to keep cells and aggregates in suspension.
    - Promote homogeneous spheroid/organoid formation.
    - Reduce shear stress compared to traditional stirring.
  - **Advantages:** Long-term viability, improved nutrient/gas exchange.
  - **Limitations:** Limited control over aggregate size and uniformity, large volume.

3D Cell Culture Devices

- **Perfusion bioreactors**
  - Devices that continuously circulate media through 3D constructs.
    - Enhance nutrient/oxygen delivery and waste removal.
    - Mimic aspects of vascularization.
  - **Advantages:** Support larger, more physiologically relevant tissue constructs.
  - **Limitations:** More complex setup and lower throughput compared to well plates.



- **Organ-on-a-chip / Microfluidic devices**
  - Microengineered chips with microchannels and microchambers for dynamic flow and 3D cell culture.
    - Recreate organ-level functions (e.g., lung alveoli, gut epithelium, liver metabolism, tumor-immune interactions).
    - Allow real-time monitoring of barrier function, drug response, and cell-cell interactions.
  - **Advantages:** High physiological relevance, precision control of microenvironment.
  - **Limitations:** Expensive, technically complex, and lower throughput.



Critical Factors in Choosing a 3D Cell Culture Method

- **Research purpose**
  - **Disease modeling** → Organoids or 3D cell culture in biomimetic hydrogels.
  - **High-throughput drug screening** → Simple, reproducible spheroid models (e.g., ULA plates).
  - **Immune cell interactions** → Dynamic systems (e.g., organ-on-a-chip).
  - **Regenerative medicine / tissue engineering** → Bioprinted tissue constructs.

- **Physiological relevance**
  - **Simple 3D cell structure** → Scaffold-free spheroids.
  - **ECM, mechanical stiffness, or growth factor signaling required** → Biomimetic hydrogels, decellularized ECMs, organoids.
  - **Gradients of oxygen/nutrients/flow** → Perfusion bioreactors, organ-on-a-chip.

- **Cell type**
  - **Pluripotent stem cells / primary cells** → Require ECM-rich environments (e.g., Matrigel for organoid generation).
  - **Cancer cell lines** → Spheroids provide efficient tumor models.
  - **Immune co-cultures** → Best supported by dynamic flow or perfusion.

Critical Factors in Choosing a 3D Cell Culture Method

- **Scalability and throughput**
  - **High-throughput evaluation** → ULA plates, pillar/perfusion plates.
  - **Small-scale, disease modeling** → Organoids, organ-on-a-chip.

- **Practical and economic considerations**
  - **Equipment** → Availability of 3D bioprinters or microfluidic devices.
  - **Cost** → Bioinks, Matrigel, and microfluidic chips are expensive.
  - **Reproducibility** → Batch-to-batch variability (e.g., Matrigel)

❖ **Conclusion:** The most critical factor in choosing a 3D cell culture method is **the balance between the required level of physiological complexity and the desired experimental throughput**, based on the specific research objective.

- **If simplicity, reproducibility, and speed are critical** → Select scaffold-free methods (e.g., ULA plates).
- **If precision, complexity, and patient relevance are critical** → Use organoids, bioprinted tissue constructs, or organ-on-a-chip.

Bioprinting® Laboratories

📌 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.

Goal: Understand the basic function of the pillar/perfusion plate platform and its intended applications.

Overview of pillar/perfusion plate technology

- Pillar geometry, hydrogel compatibility, perfusion flow mechanism
- Static vs. dynamic 3D cell culture concepts

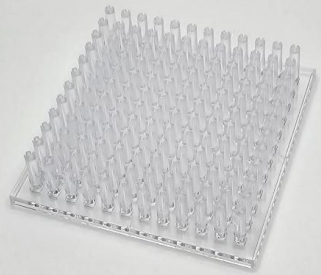
Overview of accessories: LoadingPlate and PetriLid

- Key applications:
  - ✓ 3D cell loading, encapsulation, 3D cell-based assays, imaging workflow

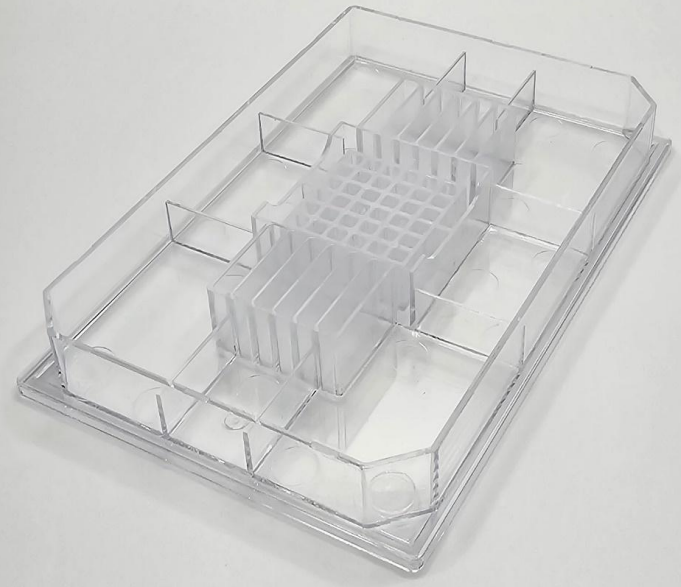
Pillar/Perfusion Plates and Accessories for Static and Dynamic Cultures of 3D Cells and Organoids

384PillarPlate

144PillarPlate



36PerfusionPlate

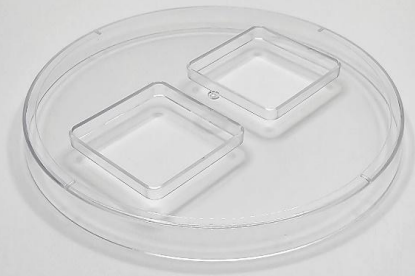


384DeepWellPlate

144PetriLid

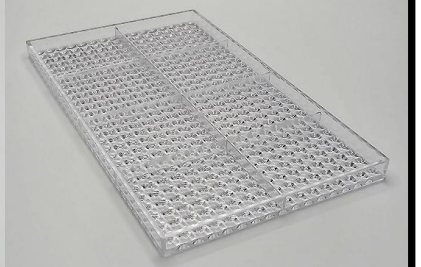
36PillarPlate

36PetriLid



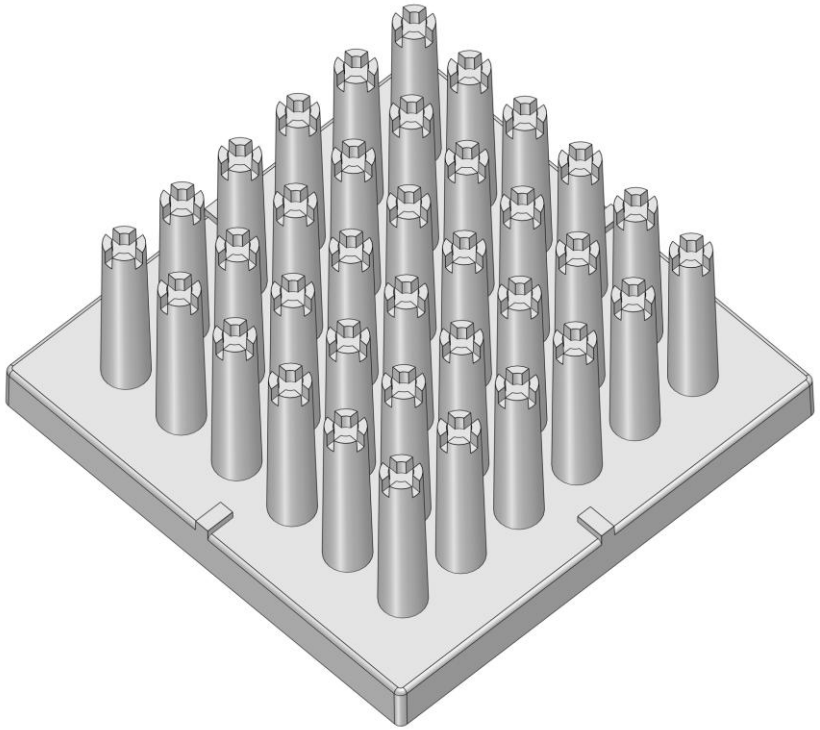
144PerfusionPlate

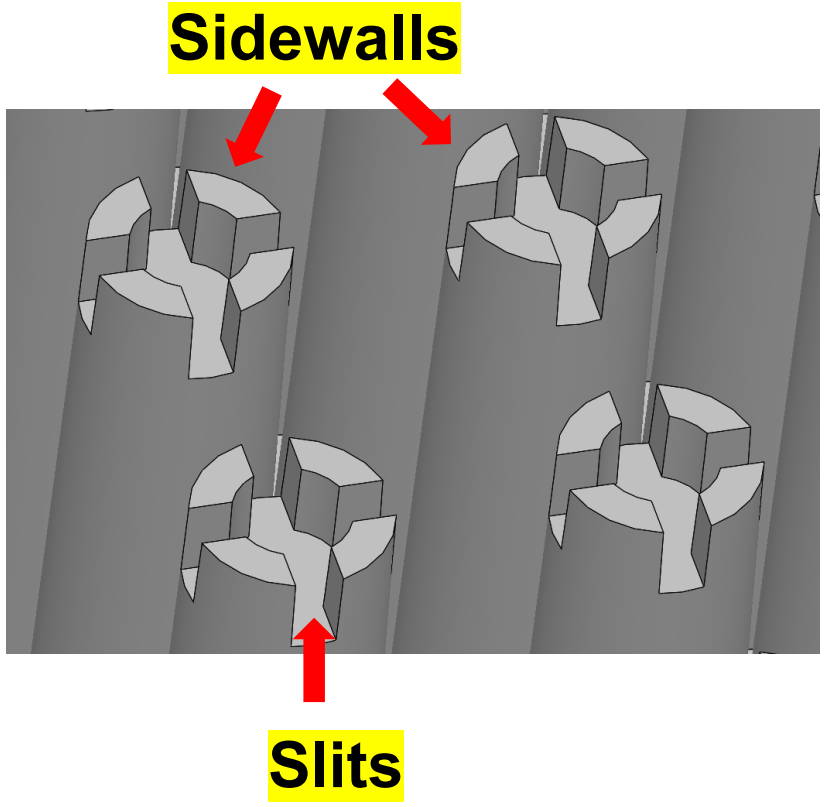
LoadingPlate



https://3dbpl.com/products/

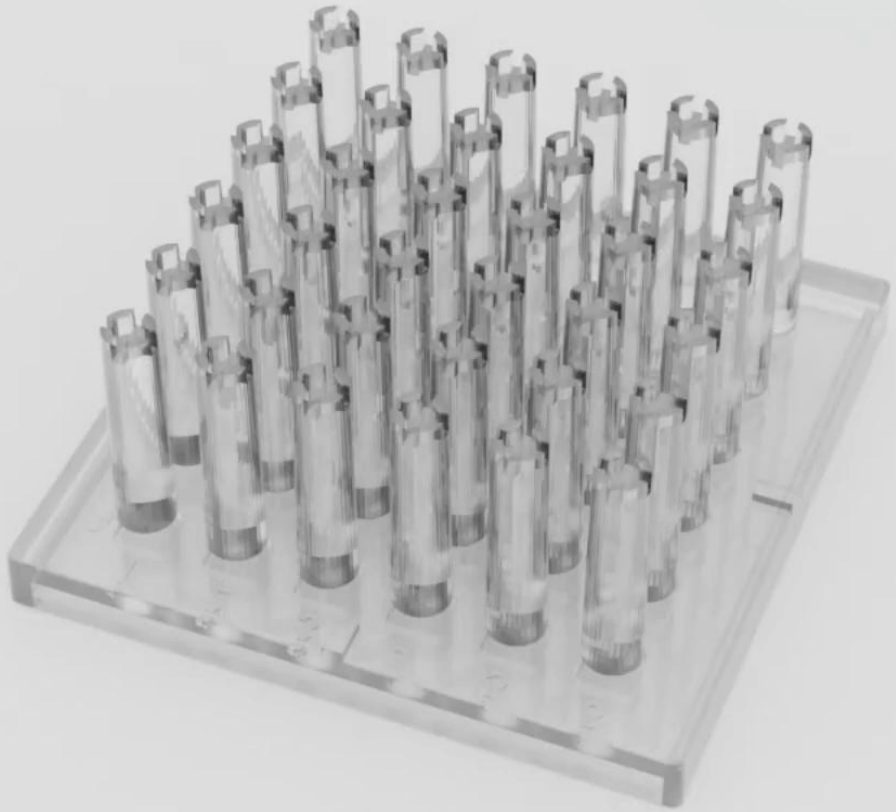
36PillarPlate



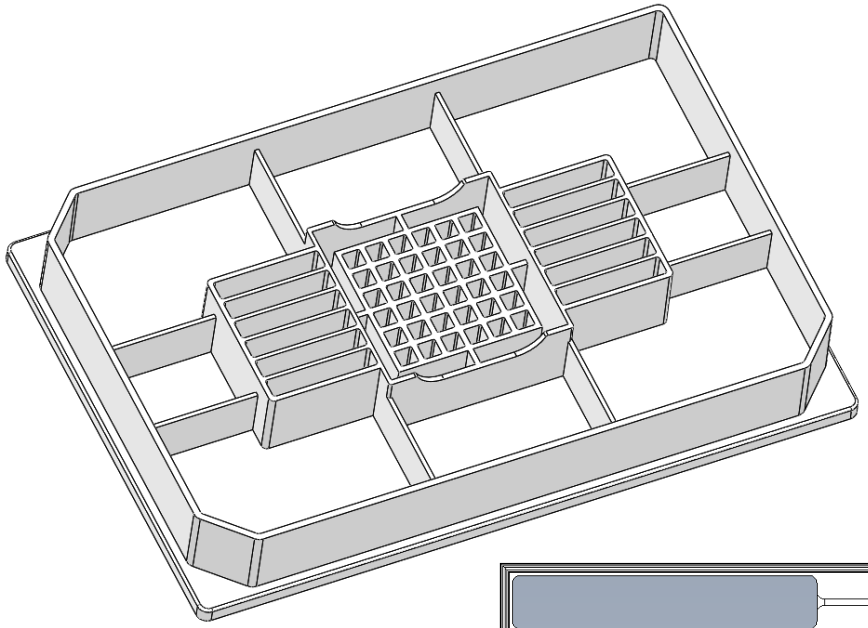


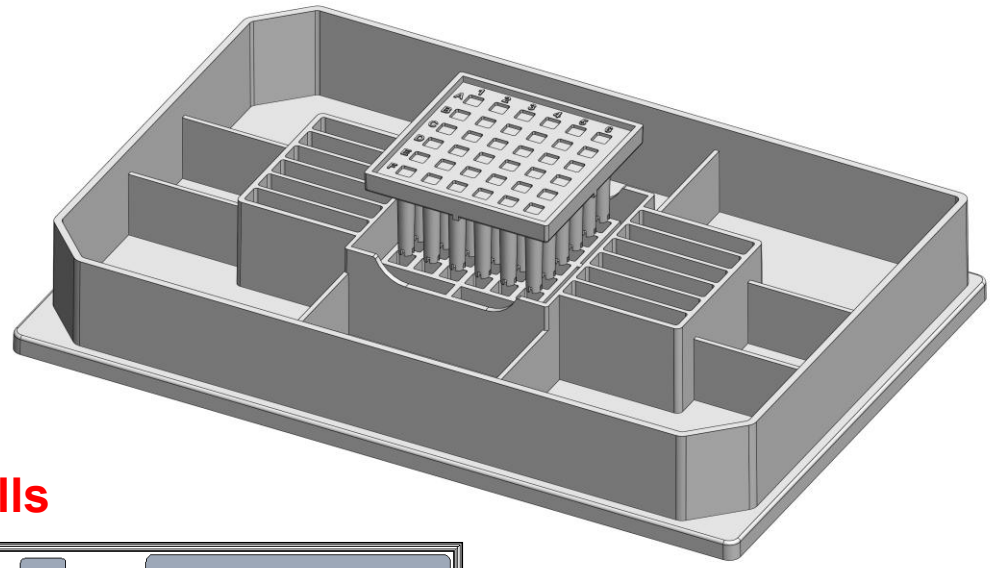
The **36PillarPlate** contains a 6 x 6 array of pillars (each pillar with sidewalls and slits) onto which suspension of single cells and spheroids in hydrogels can be loaded and cultured.

Bioprinting® Laboratories

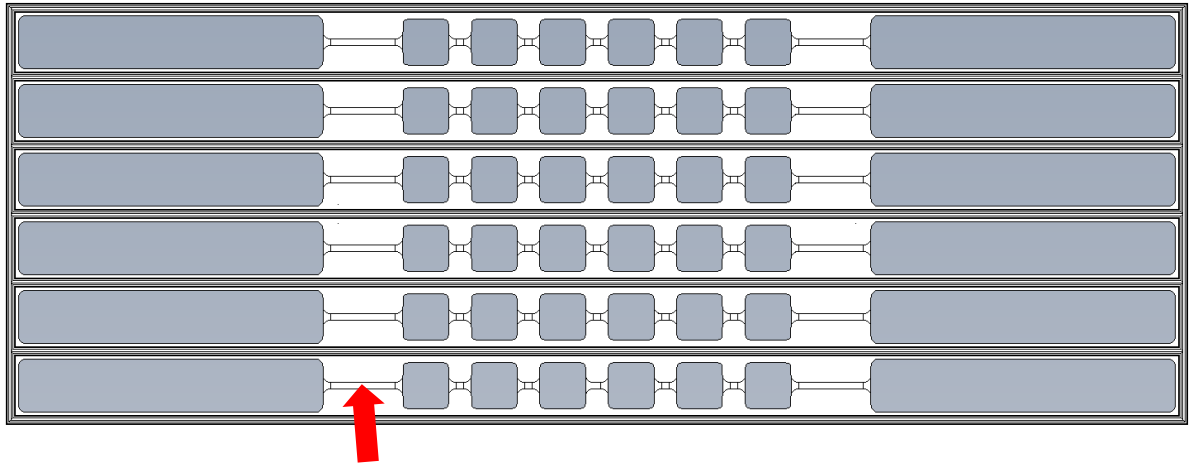


36PerfusionPlate





Perfusion wells

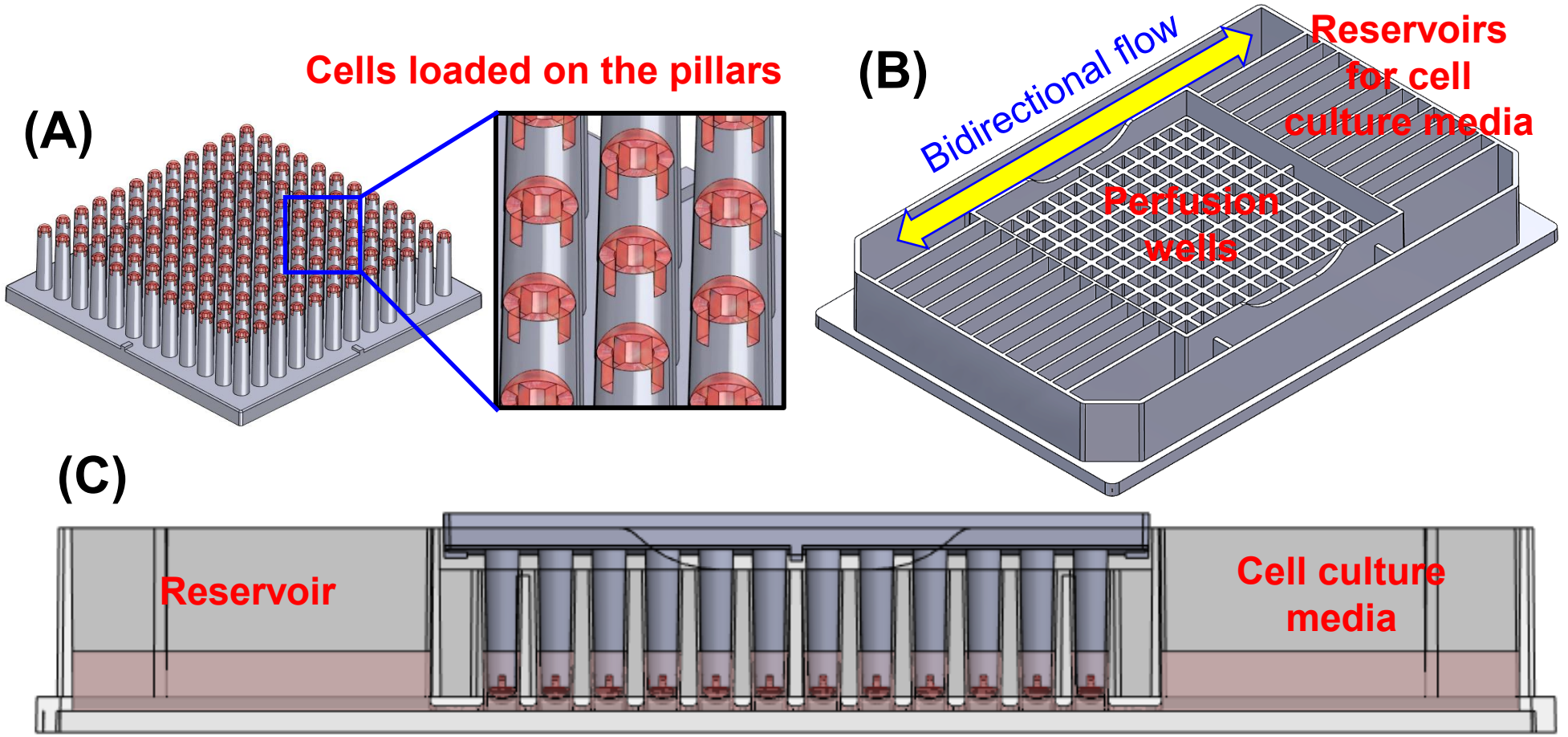


Reservoirs for cell culture media

Microchannels

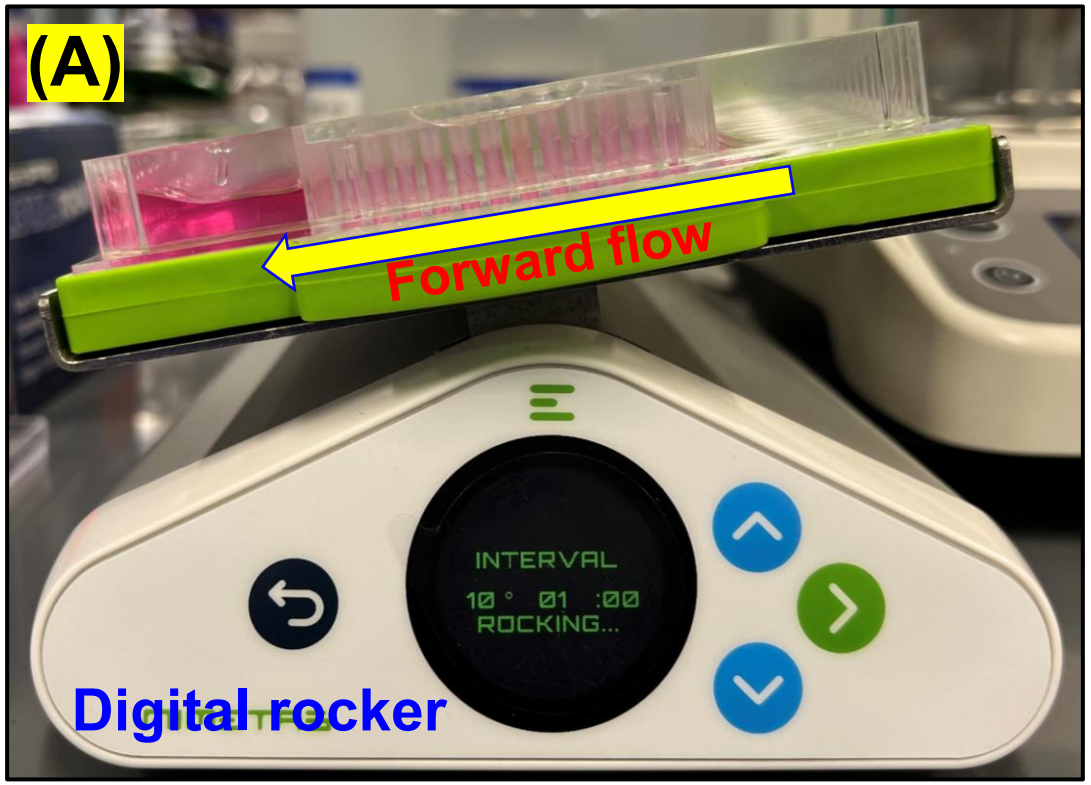
The 36PerfusionPlate contains a 6 x 6 array of perfusion wells and a 2 x 6 array of reservoirs (each channel consisting of 6 perfusion wells and 2 reservoirs).

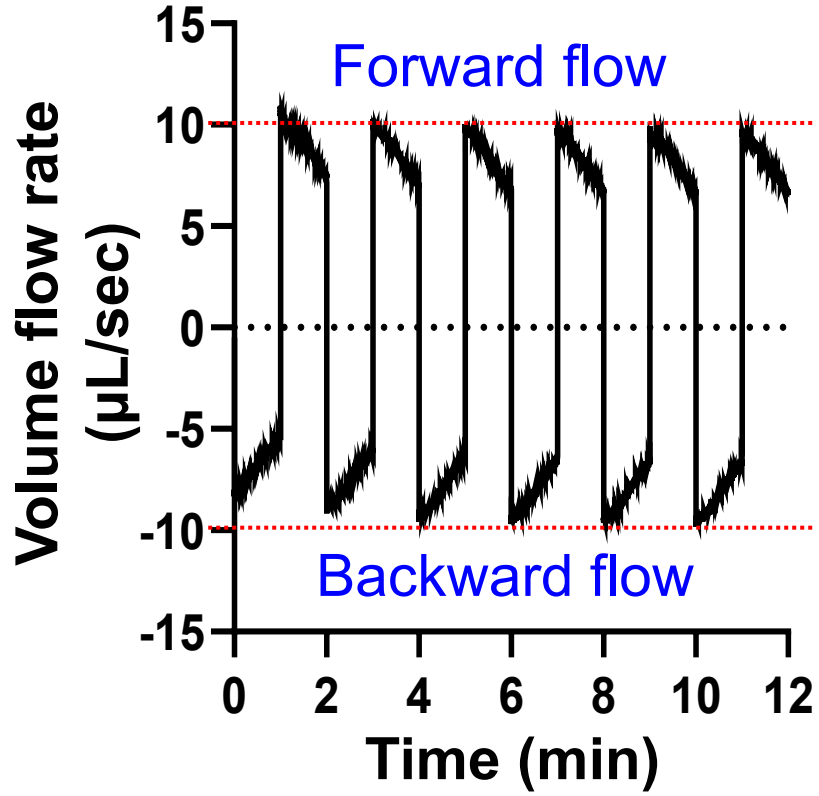
Pillar/Perfusion Plate for Dynamic Organoid Culture



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.

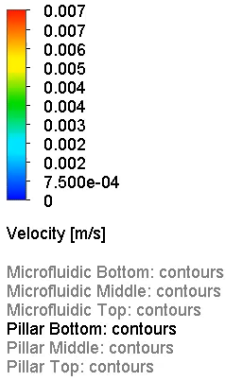
Pillar/Perfusion Plate for Dynamic Organoid Culture

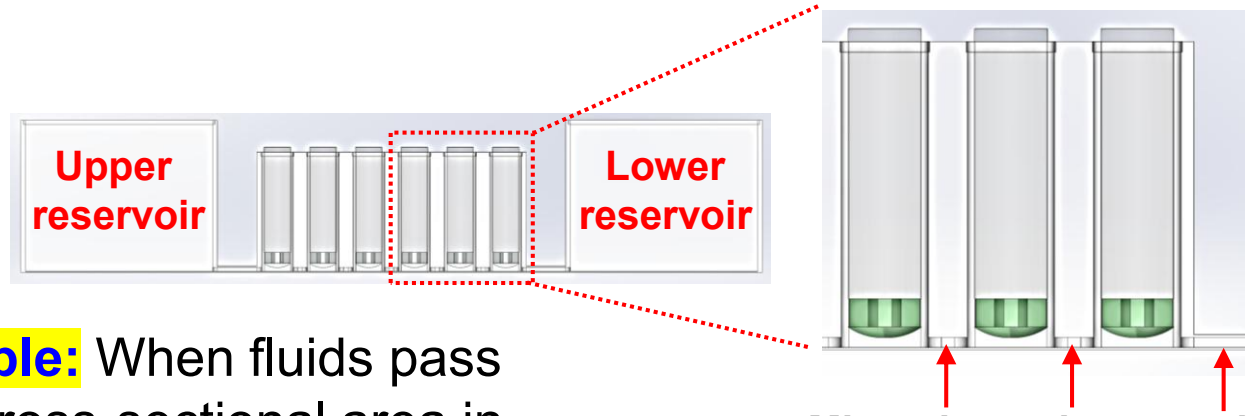




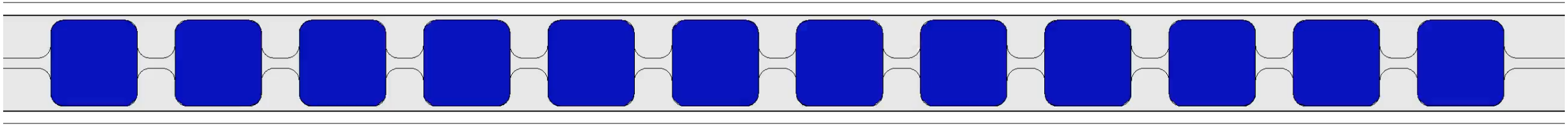
Our 144PillarPlate and 144PerfusionPlate platform. (A) The injection-molded pillar/perfusion plate on a digital rocker for dynamic organoid culture. **(B)** Flow rates in the perfusion plate with 1,500 µL culture media at 10° tilting angle and 1 minute frequency of tilting angle change. SolidWorks simulation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





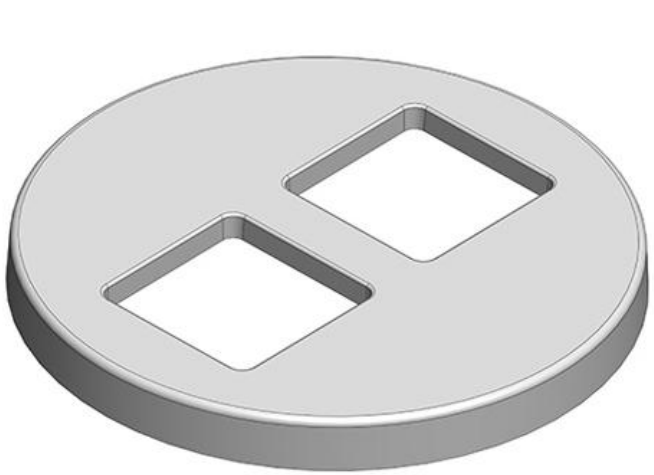
Bernoulli's principle: When fluids pass through a narrow cross-sectional area in a pipe, flow rates are accelerated.

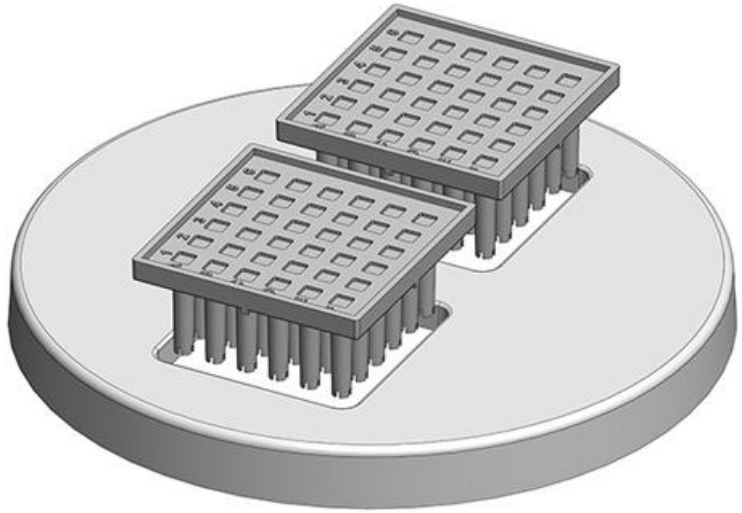


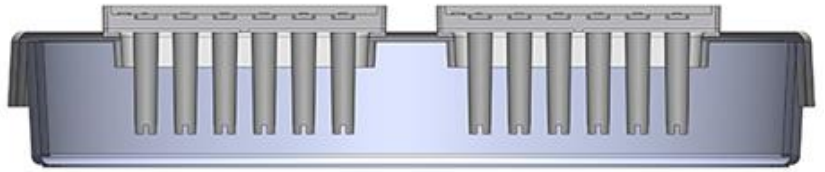
Velocity profiles underneath the pillars in the 144PerfusionPlate over time simulated with SolidWorks with 1500 µL of water at 10° tilting angle, 1 min frequency, and total 4 min running time. The unique design, where pillars are sandwiched into perfusion wells, facilitates rapid mixing of cell culture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

https://youtu.be/irLQIB6Ljnk

36PetriLid

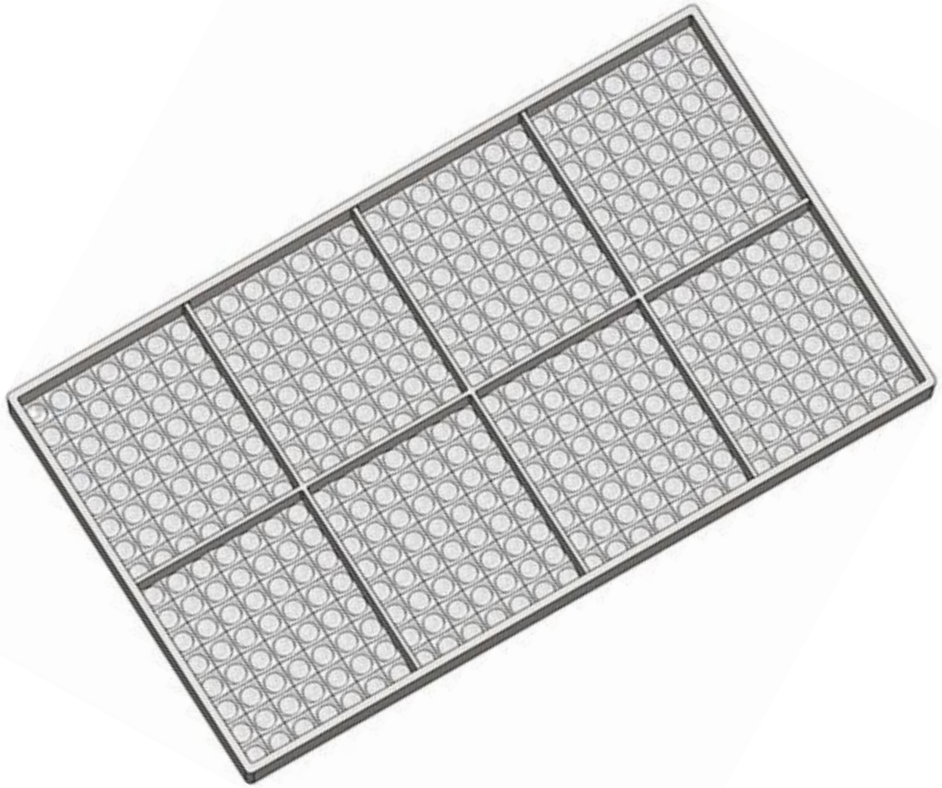


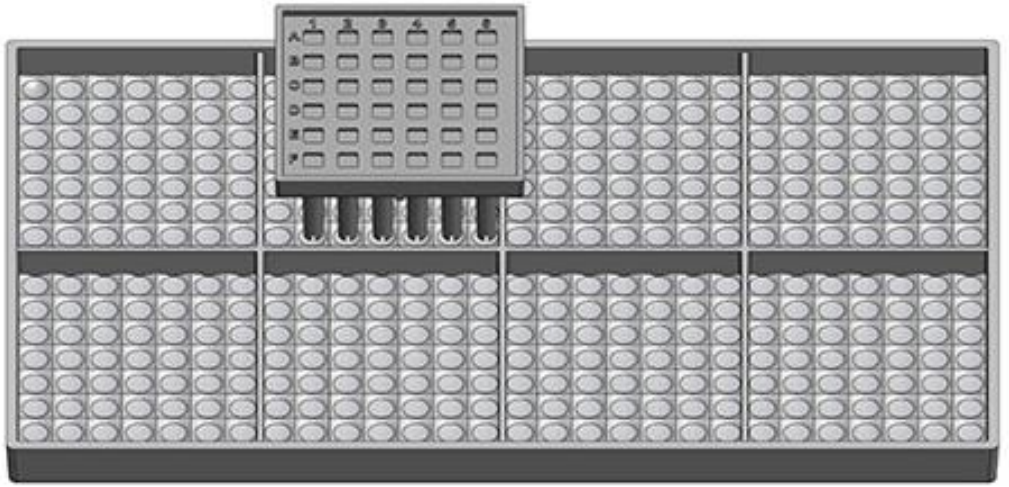




The **36PetriLid** has 2 holes for accommodating two 36PillarPlates. The **36PetriLid** is complementary to traditional petri dishes (90 mm x 15 mm) and used for static and dynamic culture of 3D cells and organoids on the 36PillarPlate as well as hydrogel gelation and immunofluorescence staining of cells.

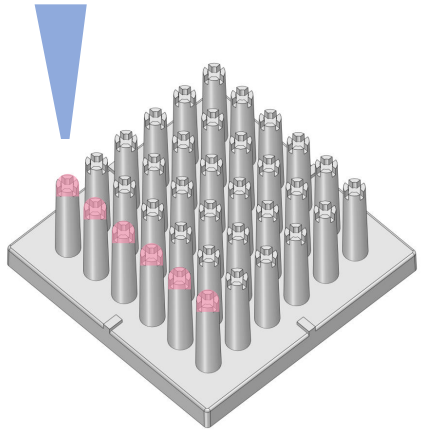
LoadingPlate



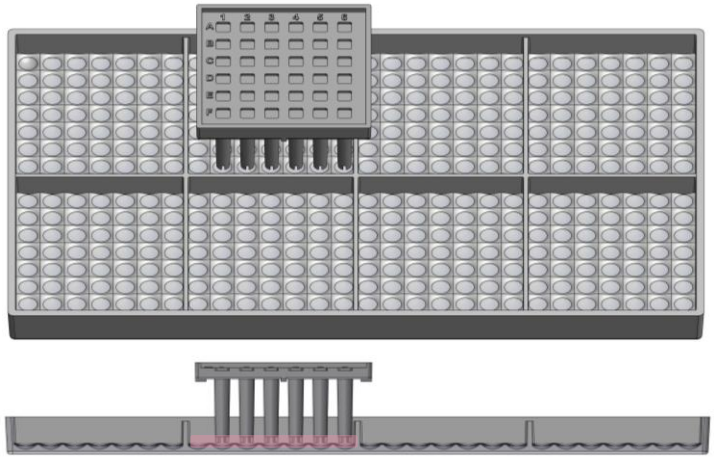


The **LoadingPlate** contains 8 blocks (each block accommodating 2 – 3 mL of hydrogels or cell suspension in hydrogels) for manual loading of hydrogels or cells on the pillar plates.

Rapid and Robust Cell Loading on the Pillar Plate

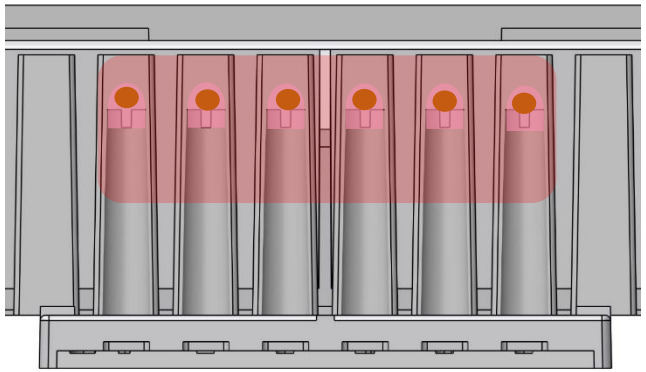


Manual dispensing with 1 mL pipette tip



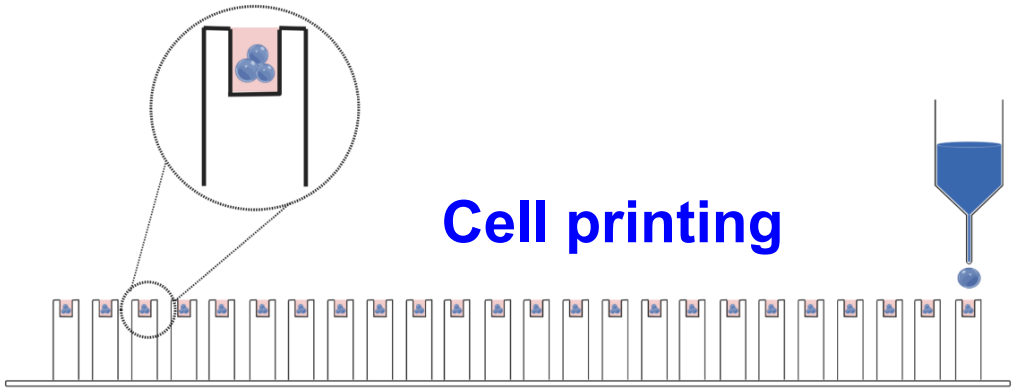
Pillar stamping

ULA 384-well plate



Spheroid transferring

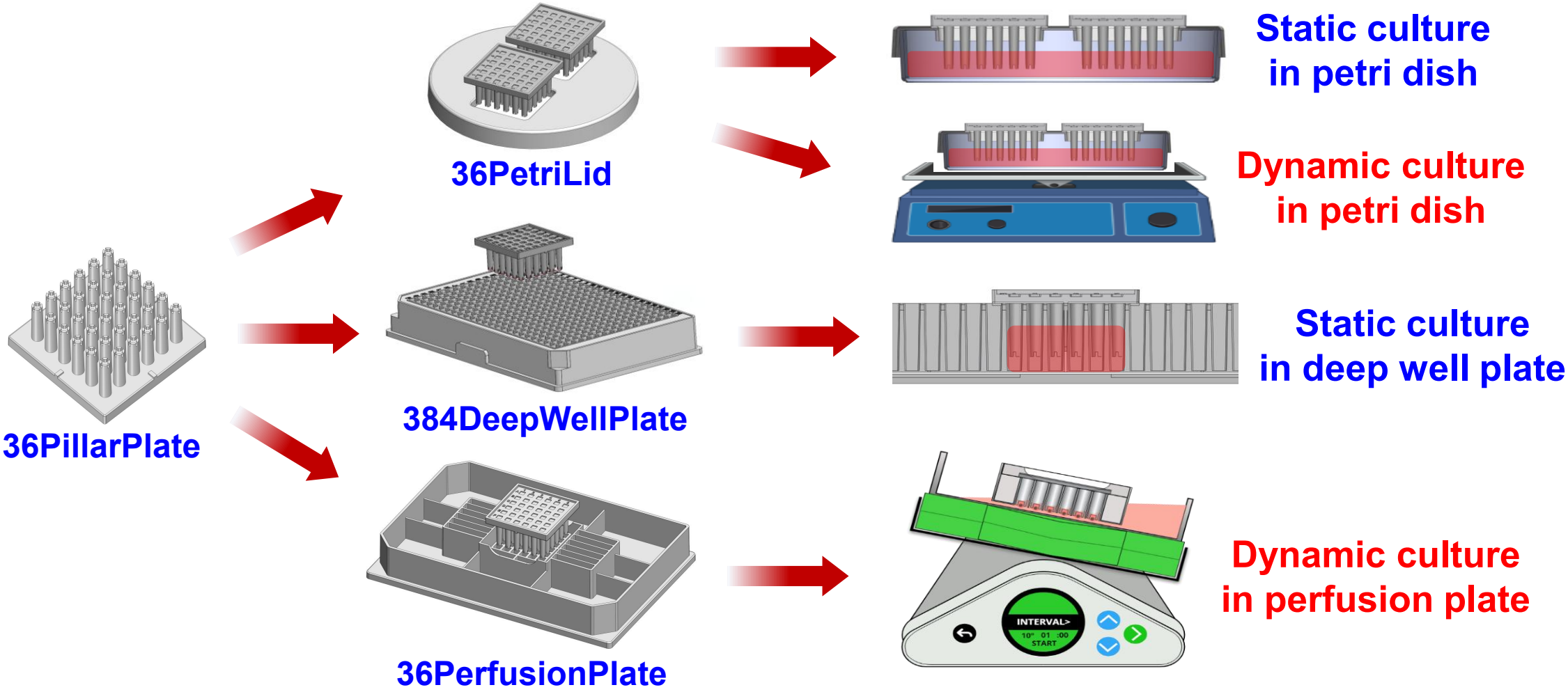
Pillar plate



Cell printing

https://youtu.be/EaDw4xoNuel

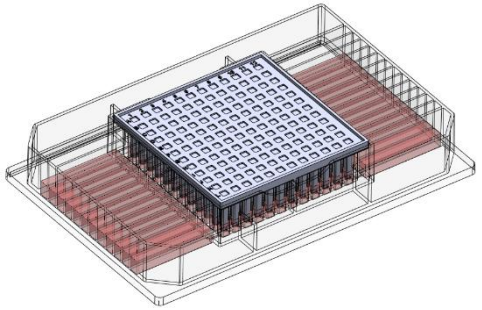
User-Friendly Organoid Culture on the Pillar Plate



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

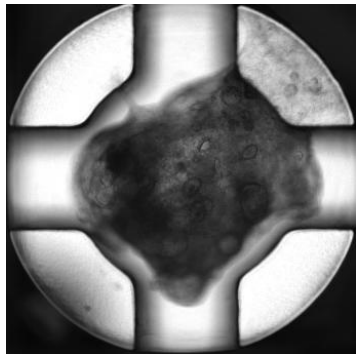
Compatible with Common Lab Equipment for Organoid Analysis

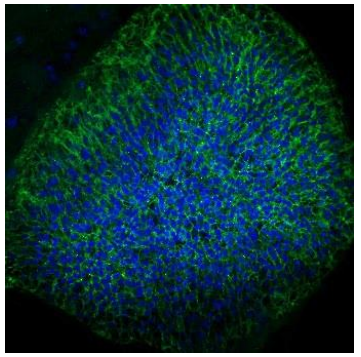


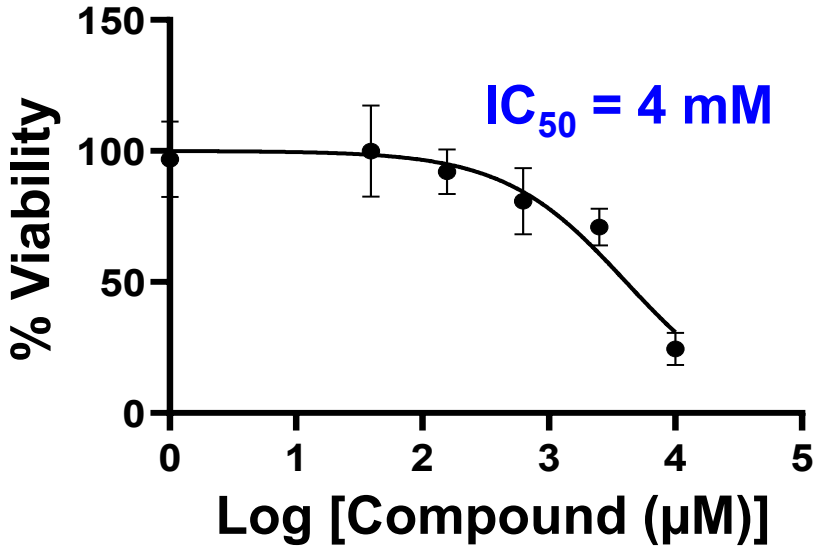
Pillar/perfusion plate

Well plate reader

Fluorescence microscope

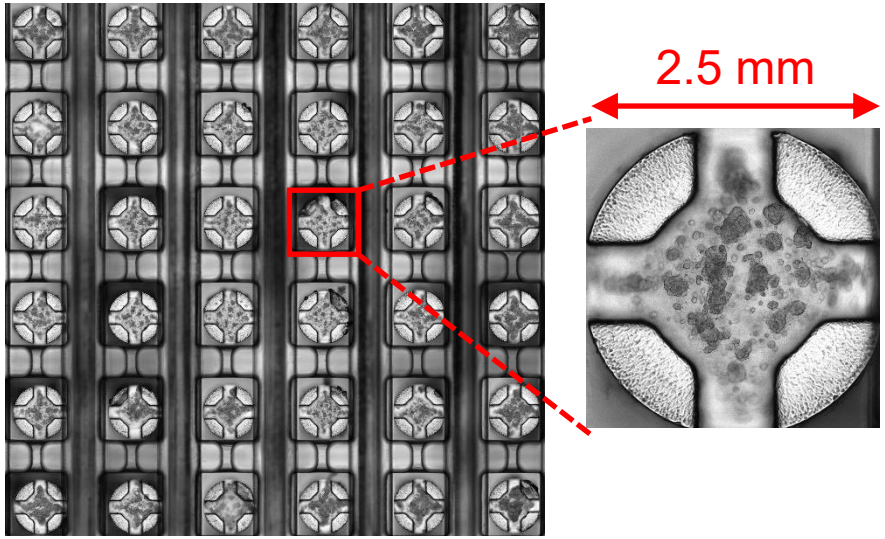




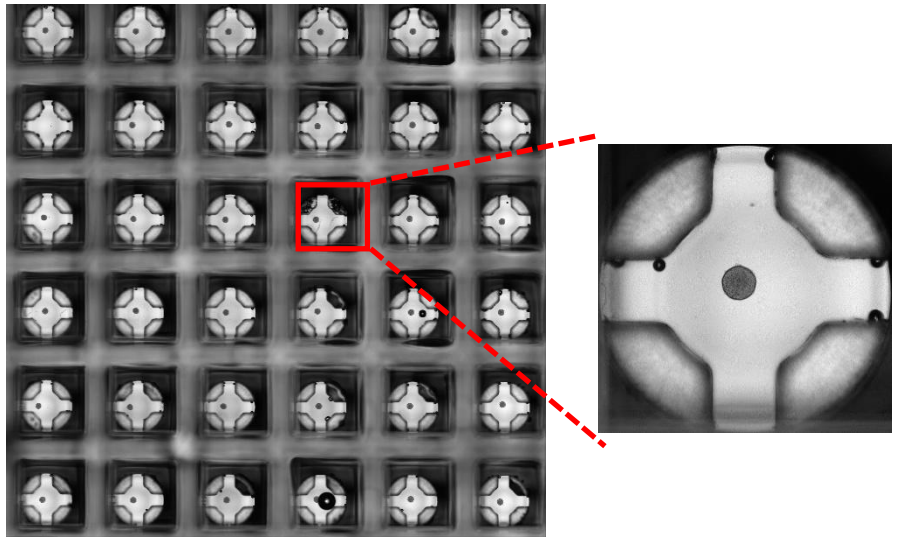


The pillar/perfusion plate is fully compatible with standard laboratory equipment for organoid analysis, eliminating the need for customers to invest in additional instrumentation.

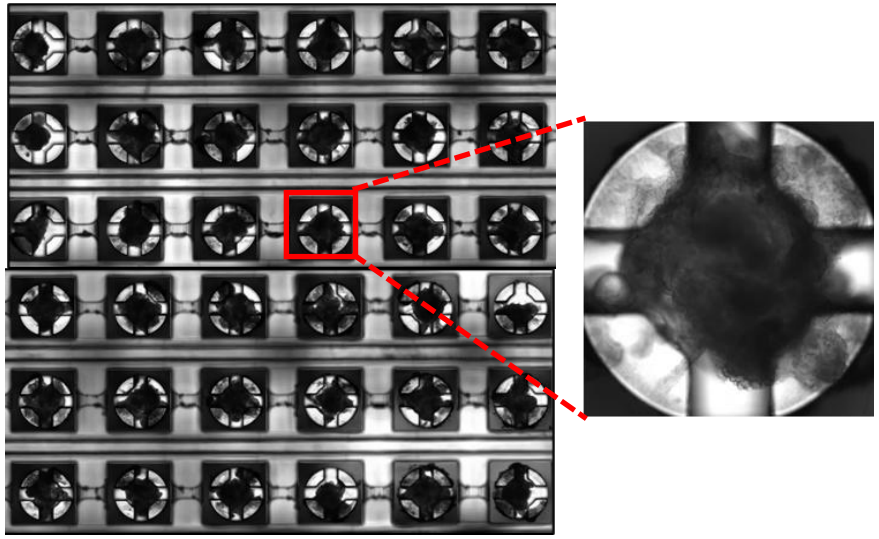
Uniformity of Organoids in the Pillar/Perfusion Plate



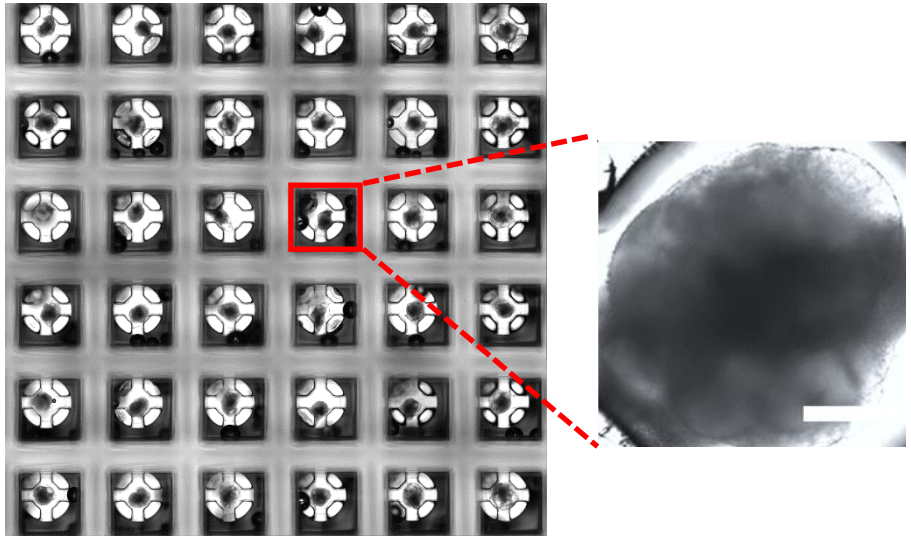
3D-cultured cancer cells



Liver cancer spheroids



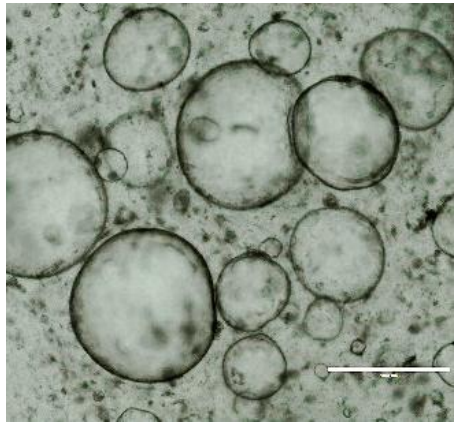
Human liver organoids



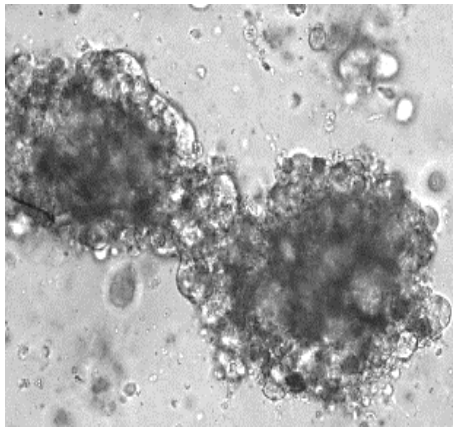
Human brain organoids

Human Organoids Created in the Pillar/Perfusion Plate

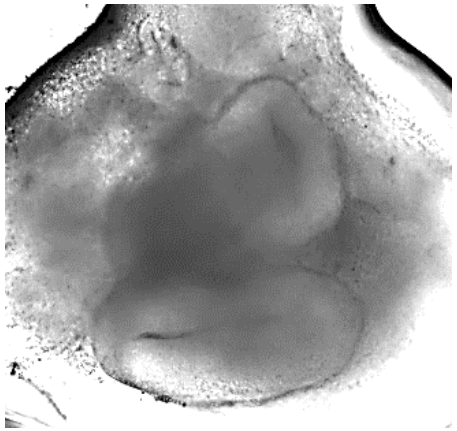
Liver



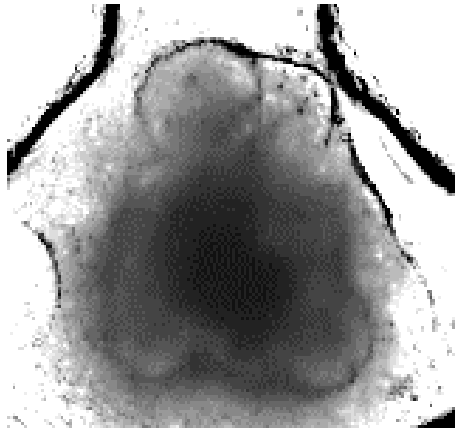
Liver tumor



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)