Bioprinting® Laboratories

# Day 2: Loading Hydrogels & Cell Suspension in Hydrogels onto Pillar Plate

www.3dbpl.com

*Innovation in Organoid Culture and Disease Modeling*

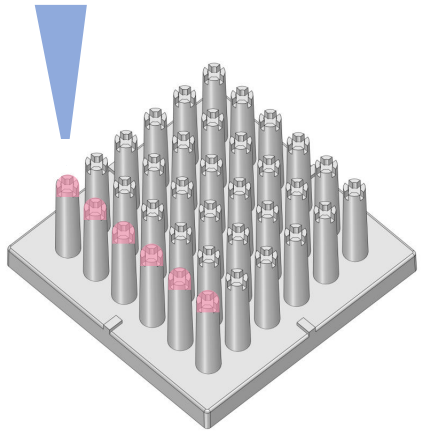
## Goal: Learn foundational skills for loading hydrogels alone and cell suspension in hydrogels onto the pillar plate.

**Introduction to hydrogels used in the workflow**

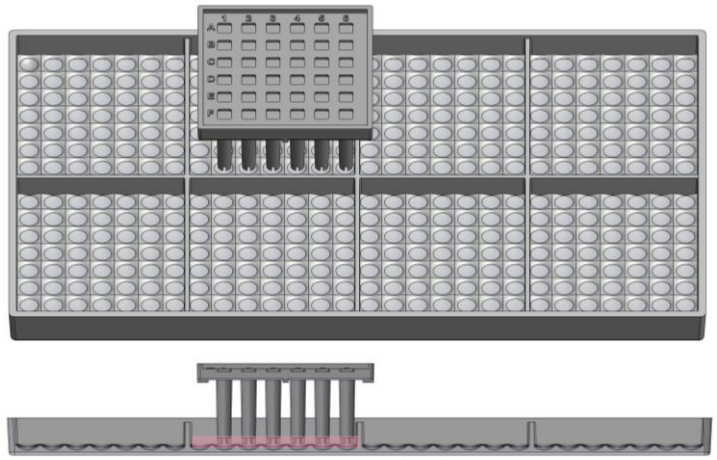
- **Matrigel, alginate-gelatin (properties, viscosities, temperature sensitivity)**
- **Preparation of Matrigel and alginate**

**Best practices for manual dispensing with a 1 mL pipette and cell loading with a LoadingPlate (pipette types, volumes, timing, avoiding bubbles)**

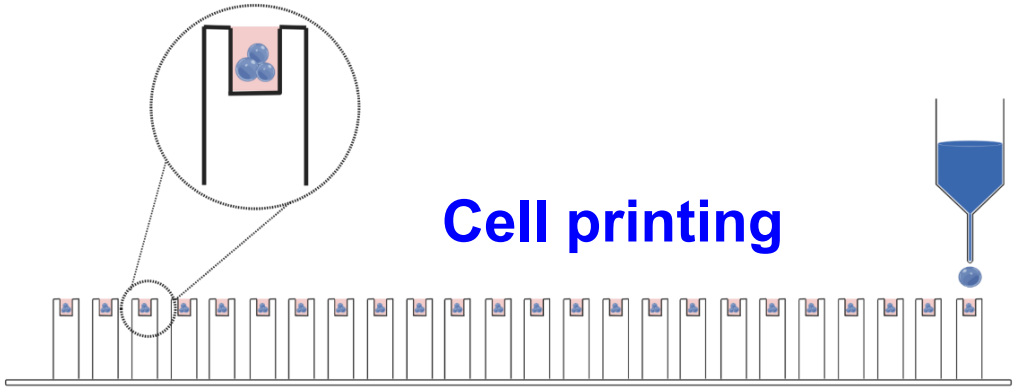
# Rapid and Robust Loading of Hydrogels and Cell Suspension in Hydrogels on the Pillar Plate



Manual dispensing with 1 mL pipette tip



Pillar stamping



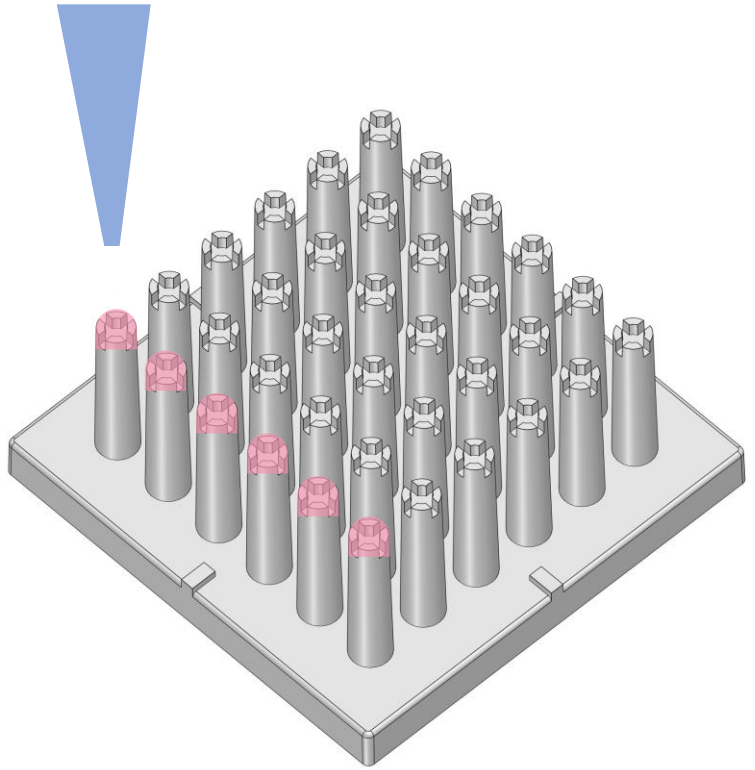
Cell printing

https://youtu.be/EaDw4xoNuel

Bioprinting Laboratories

# Manual Loading

Used for loading either single cell in hydrogel or for loading hydrogels alone onto one or two pillar plates

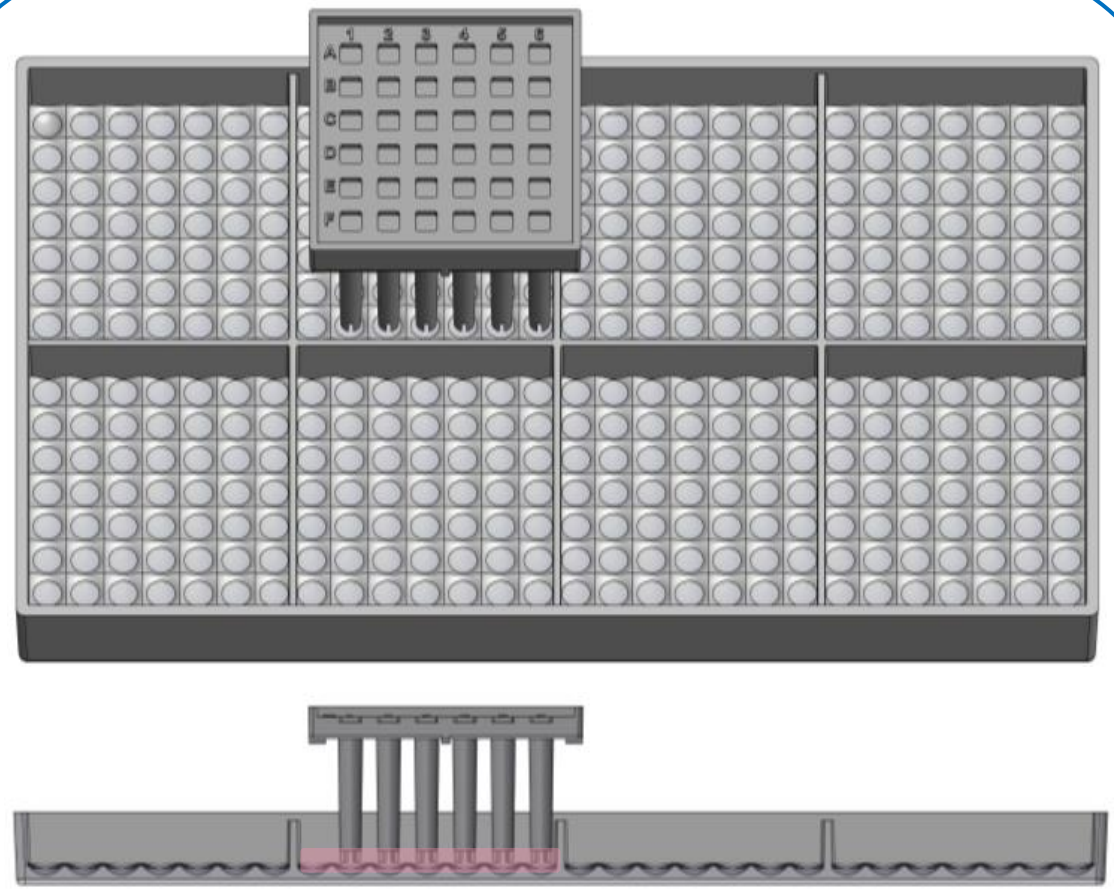


**Manual dispensing with 1 mL pipette tip**

Bioprinting Laboratories

# Pillar Stamping

Used for rapidly loading a suspension of single cells in hydrogels, or for loading hydrogels alone onto multiple pillar plates

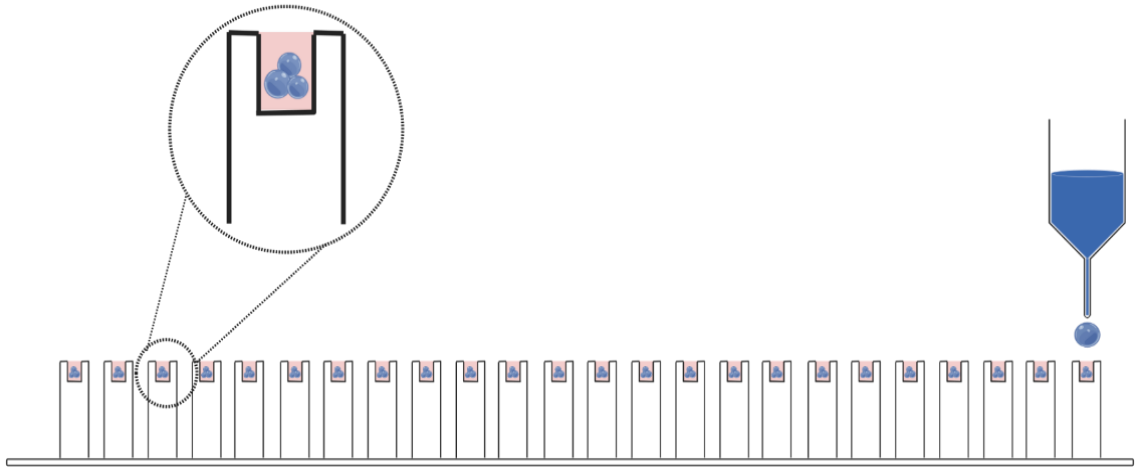


Pillar stamping

Bioprinting Laboratories

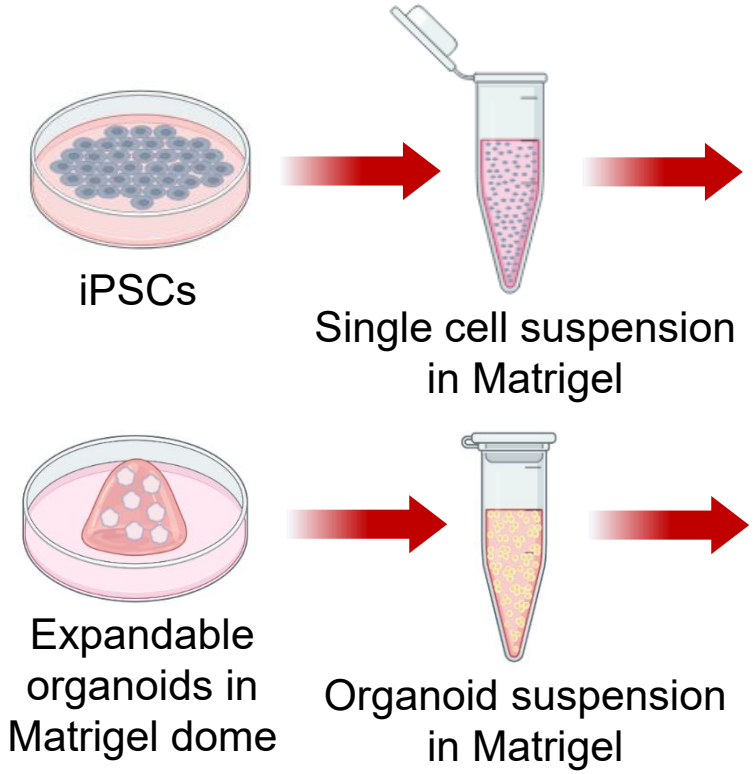
# Cell Printing

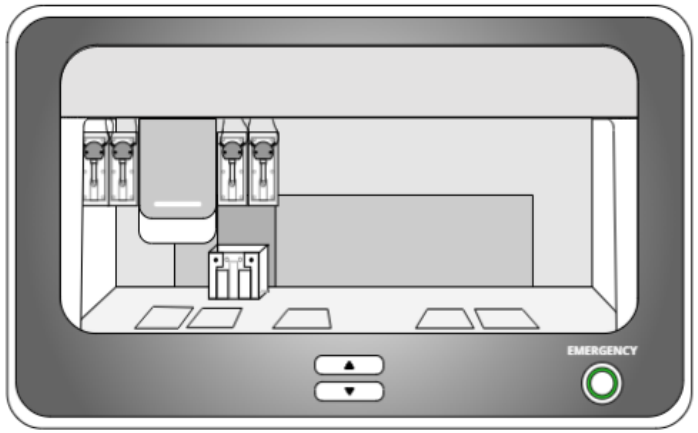
Used for rapidly loading a suspension of single cells and small spheroids in hydrogels, or for loading hydrogels alone onto multiple pillar plates for high-throughput screening (HTS)



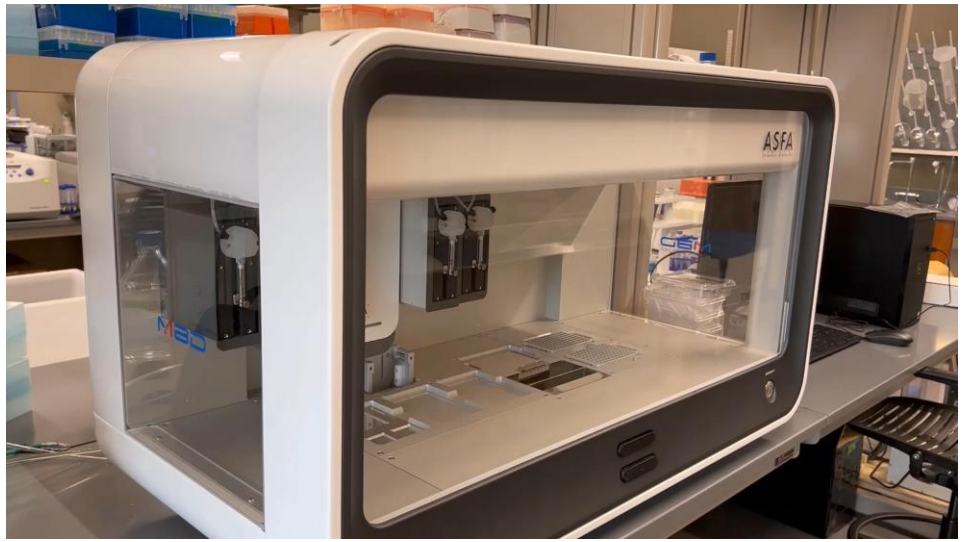
**Cell printing**

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




Bioprinting of cells on the pillar plate



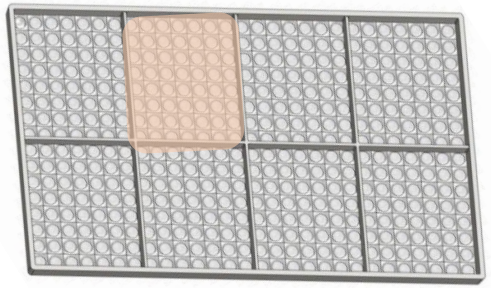

Bioprinting of single cells and spheroids suspended in hydrogels on the pillar plate using a 3D bioprinter. The typical coefficient of variation (CV) for cell printing ranges from 10% to 20%, demonstrating high reproducibility in cell loading *via* 3D bioprinting.

https://3dbpl.com/resources/

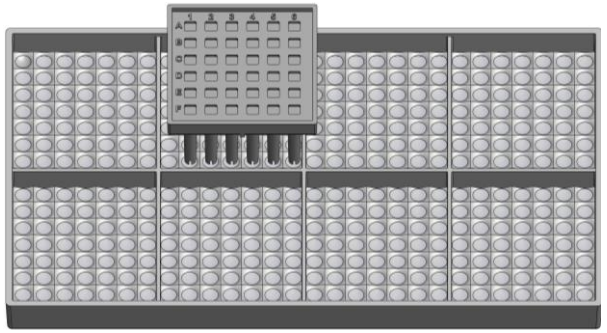
# Best Practices for Matrigel Use

- Matrigel® stock should be thawed overnight by submerging the unopened bottle in an ice bucket filled with ice in a 4°C refrigerator.
- Aliquots of 500 µL is recommended to be prepared and stored at - 20°C for future use.
- It is necessary to maintain a low temperature while loading Matrigel alone or mixing with cells to avoid premature gelation.
- With 1.5 - 2 mL of the cell-Matrigel mixture, we can prepare at least three 36PillarPlates (5 µL cell-Matrigel mixture per pillar or 180 µL the cell-Matrigel mixture per 36PillarPlate) without introducing macro-bubbles on the pillars.

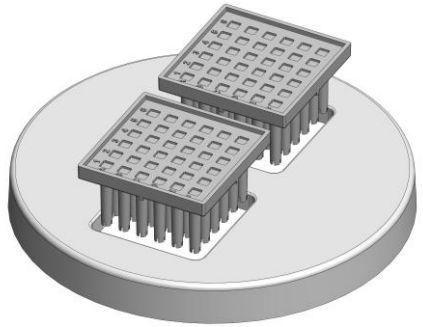
# Cell Suspension Culture in Matrigel on 36PillarPlate



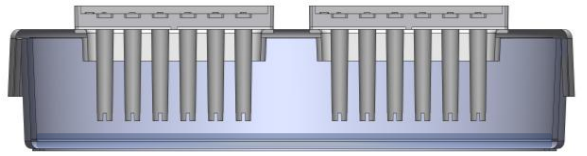
Dispense 2 mL cell-Matrigel mixture per block in the loading plate



Stamp the 36PillarPlate to load cells on pillars



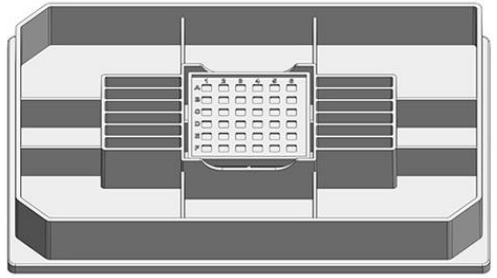
Insert the pillar plate in the 36PetriLid



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

Separate the pillar plate from the 36PetriLid

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

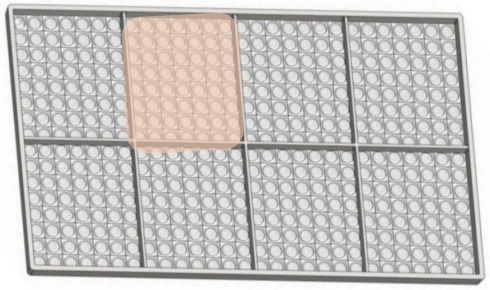


Sandwich the pillar plate onto the perfusion plate for dynamic cell culture

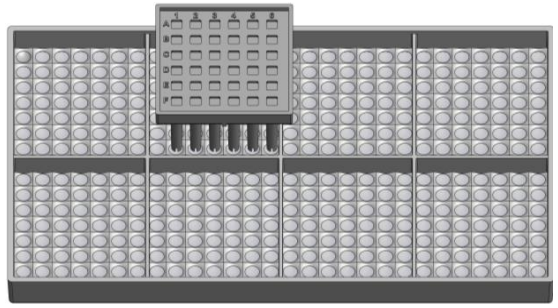
# Best Practices for Alginate + Gelatin Use

- A 2% (w/v) stock of medium-viscosity alginate should be prepared.
- Medium-viscosity alginate should be dissolved by continuously stirring for 3 days on a magnetic stirrer using a large magnetic bar for stirring.
- Use of 2% gelatin solution is recommended for the alginate + gelatin mixture.
- The 2% gelatin solidifies at 4°C and therefore must be thawed at room temperature before mixing with alginate.
- Before use, inspect the gelatin solution for any precipitate formation.
- Keep both the alginate and gelatin solutions at room temperature prior to mixing to minimize bubble formation.

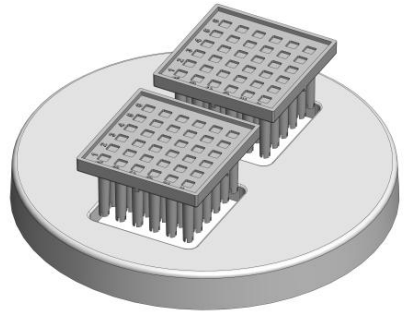
# Cell Suspension Culture in Alginate on 36PillarPlate



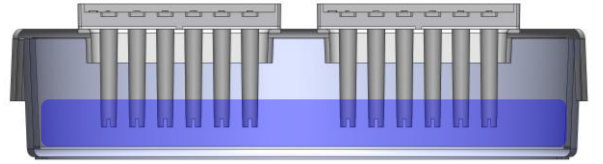
Dispense 2 mL cell-alginate mixture per block in the loading plate



Stamp the 36PillarPlate to load cells on pillars

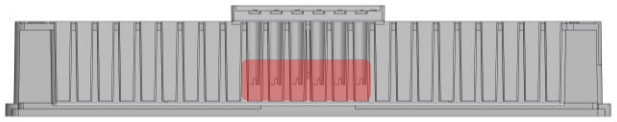


Insert the pillar plate in the 36PetriLid

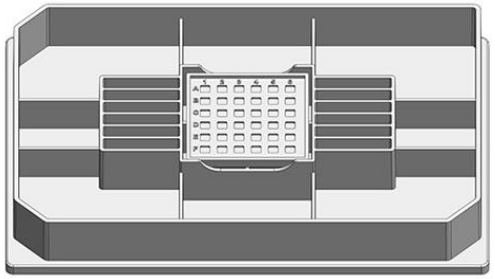


Incubate the pillar plate at room temperature for 5 mins in a petri dish with 20 mM $CaCl_2$ + 5 mM $BaCl_2$ for alginate gelation

Separate the pillar plate from the 36PetriLid



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



Sandwich the pillar plate onto the perfusion plate for dynamic cell culture

# Some Precautionary Steps to Avoid Common Errors

- Hydrating the surface of the pillar plate in a humid environment is necessary to make it hydrophilic and avoid air bubble entrapment on the pillars after cell loading.
- Use of warm cell culture medium during the mixing with cold alginate is critical to prevent air bubble entrapment on the pillars.
- Cell-hydrogel mixture should be well suspended before dispensing on the LoadingPlate to avoid non-uniform loading on the pillar plate due to cells settling down.
- Cell culture media should not be used for alginate gelation as it may cause salt precipitation. Use a saline solution instead.