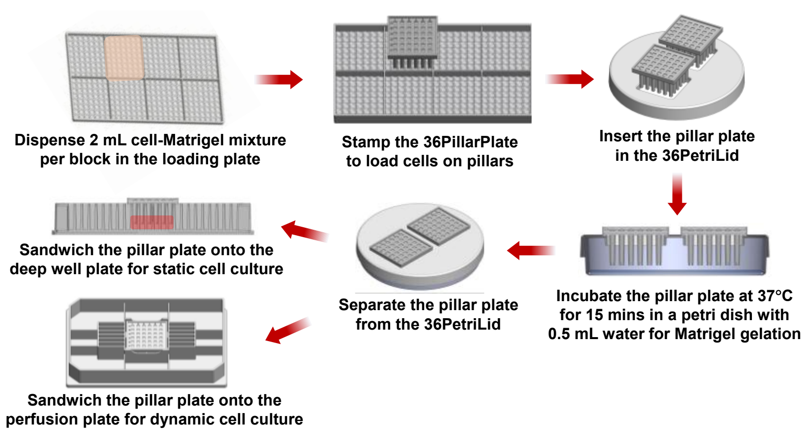
Application Note

Single Cell Suspension Culture on the Pillar Plate – A rapid and reproducible method for high-throughput 3D cell-based assays

Three-dimensional (3D) cell culture platforms such as the 36PillarPlate enable rapid generation of physiologically relevant spheroids and organoids with high reproducibility and minimal hands-on time. This application note describes standardized procedures for loading single-cell suspensions encapsulated in Matrigel® or alginate onto the pillar plate using either (i) a stamping method with the LoadingPlate or (ii) a single-tip manual loading method. The workflows summarized here are adapted from Bioprinting Laboratories Inc.'s validated standard operating procedures (SOPs) and provide practical guidance for optimizing 3D cell encapsulation, culture, and assay on the pillar plate platform.

Introduction

As the adoption of 3D cell culture continues to expand across regenerative medicine, drug discovery, toxicology, and advanced disease modeling, there is a growing need for platforms that support rapid, scalable, and reproducible generation of spheroids and organoids. Conventional microtiter well plates are often limited by inconsistent hydrogel distribution, high reagent consumption, and variability in cell seeding across replicates, which collectively compromise experimental robustness. The 36PillarPlate addresses these limitations through an array of precisely engineered pillars that support accurate deposition of cell-ECM mixtures, rapid gelation, and controlled microenvironment formation. Each plate enables the culture of 36 identical 3D microtissues, each formed from a uniform 5 µL hydrogel droplet containing a defined number of single cells. This configuration reduces reagent consumption by more than 80% compared with conventional well-plate-based 3D cultures while maintaining high experimental reproducibility. In addition, the pillar plate is fully compatible with both static and dynamic culture formats, facilitating downstream assays and scalable workflows. When paired with the LoadingPlate for high-throughput stamping or with single-tip manual loading for small-volume or precious samples, the pillar plate workflow supports seamless scaling from pilot experiments to high-throughput screening applications. Encapsulation of single cells within hydrogels is critical for recreating physiologically relevant 3D microenvironments. Matrigel® provides a basement membrane–rich scaffold well suited for epithelial, tumor, and stem cell applications, while alginate enables tunable mechanical properties through ionic crosslinking with $CaCl_2$ or $BaCl_2$. In both systems, the 36PillarPlate enables uniform cell distribution, rapid and reproducible gelation, and minimal bubble formation, thereby preserving cell viability and structural integrity. This application note presents a unified, step-by-step workflow for both Matrigel- and alginate-based single-cell loading on the pillar plate and highlights key parameters that ensure consistent, high-quality 3D cell cultures suitable for downstream biological assays.



Overview of single cell loading in Matrigel

Materials and Equipment

- 36PillarPlate (Bioprinting Laboratories Inc., Cat. no. 36-01-00)
- LoadingPlate (Bioprinting Laboratories Inc., Cat. no. 384-03-00)
- 36PetriLid (Bioprinting Laboratories Inc., Cat. no. 36-03-00)
- 384DeepWellPlate (Bioprinting Laboratories Inc., Cat. no. 384-02-00)
- Growth factor reduced Matrigel (Corning, Cat. no. 354230)
- Alginic acid (Sigma Aldrich, Cat. no. A1112)
- Calcium chloride (Sigma Aldrich, Cat. no. C7902)
- Barium chloride (Sigma Aldrich, Cat. no. B0750)
- Petri dish, 90 mm x 15 mm (VWR, Cat. no. 75799-946)
- Traditional 384-well plate (Fisher Scientific, Cat. no. 12-565-506)

Methods

(1) Pillar plate preparation: Prior to loading single-cell suspensions in hydrogel, the pillar plate surface must be properly hydrated to minimize microbubble formation and ensure consistent hydrogel deposition. Hydration is performed by placing the pillar plate in a 36PetriLid positioned on a Petri dish containing a small volume of sterile water, followed by incubation at 37°C for approximately 30 minutes. This step promotes surface hydrophilicity and improves reproducibility during pillar plate stamping. In parallel, cell culture medium should be pre-warmed and dispensed in advance into Petri dishes, 384DeepWellPlates, or 36PerfusionPlates, as appropriate. Pre-dispensing warm medium reduces bubble formation during plate assembly and subsequent culture.

(2) Preparation of single-cell suspension in hydrogel: Single-cell suspensions are prepared by gently resuspending 0.3 - 4 × 10^6 cells in warm culture medium. The cell suspension is then mixed with either chilled Matrigel or a 3% (w/v) alginate solution, depending on the extracellular matrix (ECM) selected. For Matrigel-based encapsulation, the final Matrigel concentration is typically 70 - 100%, and all handling steps must be performed at low temperature (≤ 10°C) to prevent premature gelation. For alginate-based encapsulation, the final mixture typically contains 1% (w/v) alginate, which supports efficient ionic crosslinking during gelation. In both cases, it is critical to work efficiently, as cell settling can occur within approximately 5 minutes, potentially resulting in non-uniform cell distribution across pillars.

(3) Cell loading onto the pillar plate: For rapid and uniform cell loading, the cell-hydrogel mixture is dispensed into the LoadingPlate, and the hydrated pillar plate is gently stamped to deposit approximately 5 µL of the mixture onto each pillar. Using 2 mL of cell suspension in hydrogel, at least three 36PillarPlates can be prepared. When cell samples are limited, a single-tip manual loading method may be used, in which a pipette tip containing the cell-hydrogel mixture is gently tapped to dispense individual droplets onto each pillar.

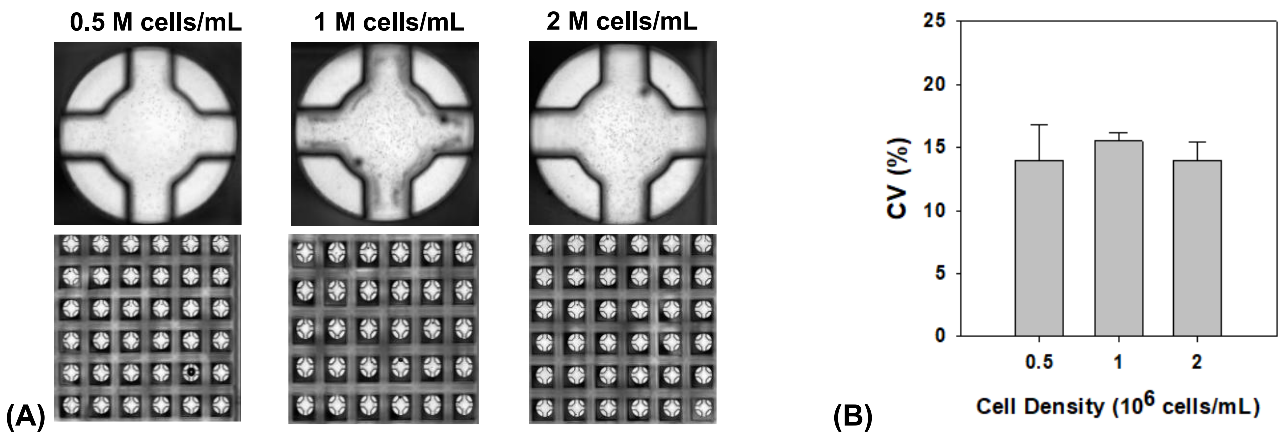
(4) Hydrogel gelation and culture: Following cell loading, Matrigel gelation is achieved by incubating the pillar plate at 37°C for 15 minutes in a humidified chamber. For alginate gelation, the pillar plate is immersed in a solution containing 20 mM $CaCl_2$ and 5 mM $BaCl_2$ for 5 minutes to induce ionic crosslinking. Once gelation is complete, the pillar plate containing encapsulated cells is assembled ("sandwiched") with a 384DeepWellPlate, 36PerfusionPlate, or Petri dish, depending on the intended culture format. Cultures are maintained at 37°C under standard incubation conditions with regular medium changes as required for the specific cell type and assay.

Results

Uniform loading of single-cell suspensions within hydrogel droplets on the pillar plate resulted in highly consistent microtissue formation across all 36 pillars. Proper hydration of the pillar surface significantly reduced bubble entrapment, enabling smooth and continuous distribution of single cells within each hydrogel droplet. Microscopic imaging confirmed homogeneous cell encapsulation with minimal pillar-to-pillar variability in droplet size and cell density. For alginate-based encapsulation, ionic crosslinking generated stable, spherical hydrogel droplets that retained structural integrity throughout the culture period. In contrast, Matrigel-based droplets exhibited a characteristic dome-shaped morphology following thermal gelation, consistent with basement membrane-derived ECM behavior.

Across multiple experiments and a range of initial cell densities, the pillar plate supported high cell viability and reproducible spheroid organization. Cells encapsulated in Matrigel demonstrated rapid spreading and interaction with the ECM, whereas cells encapsulated in alginate maintained a more rounded morphology, reflecting physical confinement within the hydrogel network. In both hydrogel systems, occasional microbubbles observed at droplet edges dissipated within 1 - 2 days of culture without compromising microtissue formation.

The pillar plate platform supported both static and dynamic culture conditions. Dynamic culture using low-speed rocking enhanced nutrient and oxygen transport to the microtissue core, resulting in accelerated growth and improved uniformity for most cell types compared with static culture.



Reproducible loading of Hep3B cells on the pillar plate. (A) Hep3B cells suspended in 0.75% (w/v) alginate were loaded onto the pillar plate by manual stamping using the LoadingPlate. Three cell seeding densities (0.5, 1, and 2 × 10^6 cells/mL) were prepared and dispensed into the LoadingPlate (1.5 mL per well) prior to stamping. Alginate droplets deposited on the pillar plate were crosslinked by incubation in 20 mM $CaCl_2$ using the PetriLid for 5 minutes. **(B)** Coefficient of variation (CV) calculated from cell viability measurements using the CellTiter-Glo® assay. CV values remained below 15% across all seeding densities, demonstrating high reproducibility of cell loading on the pillar plate.

Conclusions

The 36PillarPlate provides a robust, scalable, and highly reproducible platform for 3D single-cell suspension culture using either Matrigel or alginate. By integrating pillar surface hydration, precise and uniform cell loading *via* the LoadingPlate, rapid hydrogel gelation, and compatibility with both static and dynamic culture conditions, the platform enables generation of uniform microtissues with minimal reagent consumption and reduced process variability. The consistent cell distribution observed across pillars demonstrates the platform's suitability for high-throughput 3D applications, including spheroid and organoid formation, compound screening, cell viability assays, and long-term, multi-day culture studies. As laboratories increasingly transition toward physiologically relevant 3D cell models, the pillar plate offers a practical, efficient, and scalable solution for both routine and advanced microtissue generation workflows.