Bioprinting with the Corning® Matribot® Bioprinter for High Throughput 3D Cell Culture using Corning Matrigel® Matrix

Application Note

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Introduction

In vitro cell cultures and animal experiment models are crucial instruments in basic research and preclinical studies[1]. 3D cell culture involves expanding cells in a volumetric space as aggregates, spheroids, or organoids. This technique creates a more accurate *in vitro* environment and provides an alternative to that used *in vivo* for fundamental cell biology and physiology research[2]. The complexities of assay development and challenges associated with validating and transferring assays into high throughput modes are well known bottlenecks in the drug discovery process, especially for those using 3D cell culture models[3]. Hence, 3D bioprinting is emerging as a promising technology for high throughput handling of extracellular matrices that support cell growth and differentiation into 3D structures[4].

The Corning Matribot bioprinter can dispense and print bioinks and extracellular matrices such as Corning Matrigel matrix and Collagen. It enables fast, accurate, and high throughput bioink handling for 3D cell culture with promising applications in medical research, drug discovery, toxicity testing, and other pre-clinical studies.

Materials and Methods

Printing with Corning Matrigel matrix

Corning Matrigel matrix (Corning 354230) was drawn up into a syringe and the syringe was inserted into the printhead of a Corning Matribot bioprinter (Corning 6150). The bioprinter dispensed droplets of Matrigel matrix at 9 mg/mL using Corning DNA Studio software with the parameters shown in Table 1. A single droplet printing pattern was selected for each well of a 24-well plate or a 96-well microplate (Figures 1A and 1C) while a "droplet array" was selected to dispense four droplets in each well of a 24-well plate (Figure 1B). The plates were then placed at 37°C for 15 minutes to facilitate polymerization of Matrigel matrix.

NOTE: More details on operating the Corning Matribot bioprinter can be found in the Corning Matribot Bioprinter Instruction Manual (CLS-AN-641DOC) and Corning Matribot Bioprinter Matrigel Matrix Protocol (CLS-AN-645).

Printing with cell suspensions

A549 and MDCK cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Corning 10-013-CV) with 10% fetal bovine serum (FBS, Corning 35-081-CV) until reaching 90% confluence. The medium was removed by aspiration, cells were washed with phosphate buffered saline (PBS, Corning 21-040-CV) and dissociated into single cells using 0.25% trypsin-EDTA (Corning 25-053-CI). An equal volume of complete medium (DMEM + 10% FBS) was added to the cells and mixed. The suspension was centrifuged at 1,000 rpm for 3 minutes to pellet the cells. The supernatant was discarded, and the pellet was resuspended with undiluted Matrigel matrix (Corning 356231) and the final cell density was adjusted to 50 cells/µL. The mixture of Matrigel matrix and cells was transferred into the syringe, inserted into the printhead of the bioprinter, and dispensed using the parameters stated in Table 2. The plates were incubated at 37°C for 15 minutes followed by the addition of 1 mL complete medium to each well. The plates were incubated at 37°C and 5% CO_2, and the medium was exchanged every 2 to 3 days.

Mouse intestinal organoids (STEMCELL Technologies 70931) were thawed, cultured, and passaged in complete IntestiCult™ organoid growth medium (STEMCELL Technologies 06005) according to the manufacturer's instructions until a cell density of approximately 150 organoids per well was achieved. A pre-wet 1000 µL pipet was used to break up the organoids by pipetting up and down twenty times, followed by centrifugation at 290 x g for 5 minutes at 4°C. The supernatant was carefully discarded, and the pellet was resuspended in undiluted Matrigel matrix by pipetting up and down ten times. The Matrigel matrix mouse intestinal organoid suspension was transferred into the syringe, inserted into the printhead of the bioprinter, and dispensed using the parameters stated in Table 2. The plates were incubated at 37°C for 15 minutes followed by the addition of 1 mL complete IntestiCult organoid growth medium to each well, and the medium was exchanged three times per week.

Results and Discussion

Corning Matribot bioprinter enables fast, accurate, and high throughput Corning Matrigel matrix dispensing

Corning Matrigel matrix was dispensed into each well of a 24-well plate by the Corning Matribot bioprinter (Figure 2A). This automated method is faster and more uniform compared to manual dispensing which has higher technical requirements. Printing patterns using several droplets per well improves the cellular utilization rate of the culture medium (Figure 2B), while printing individual droplets in 96-well microplates is designed for high throughput assays (Figure 2C).

Table 1. Printing parameters for dispensing Corning® Matrigel® matrix droplets using different patterns.

Printhead Parameters			
Printhead settings	Corning Matrigel matrix (9 mg/mL)		
Vessel selection	24-well plate	24-well plate	96-well microplate
Droplet pattern per well	Single droplet	Four droplets	Single droplet
Droplet volume	10 µL	5 µL	3 µL
Temperature of printbed	37°C		
Temperature of printhead	4°C		
Extrusion rate	20 µL/s		
Extrusion volume	12 µL	7 µL	5 µL
Retract volume	2 µL	2 µL	2 µL
Z-offset	0.3 mm		
Advanced			
Extra preflow volume	0 µL		
Retract rate	5 µL/s		
Postflow stop time	0 s		
Z-lift between wells	30 mm		

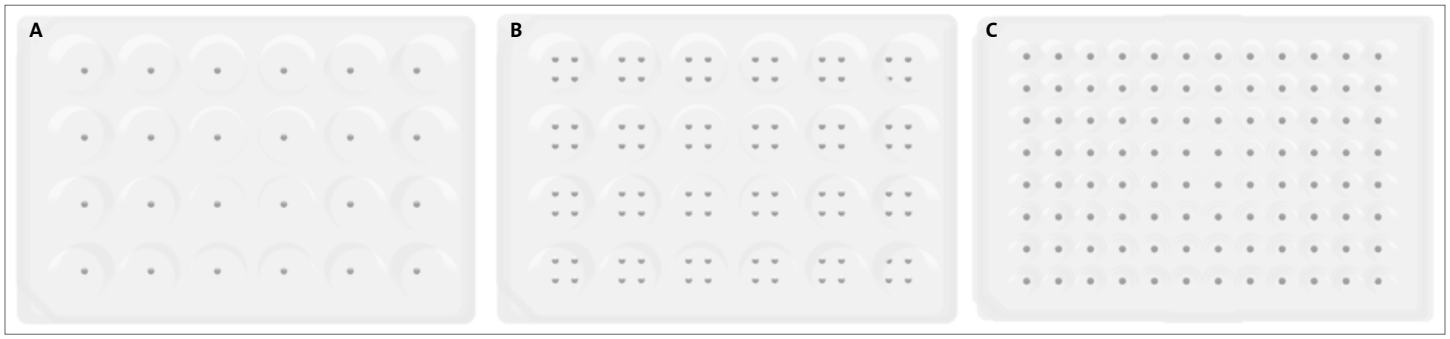

Figure 1. Different printing patterns. (A) Single droplet per well in a 24-well plate. (B) Multiple droplets per well in a 24-well plate using a square pattern of four droplets. (C) Single droplet per well in a 96-well microplate.

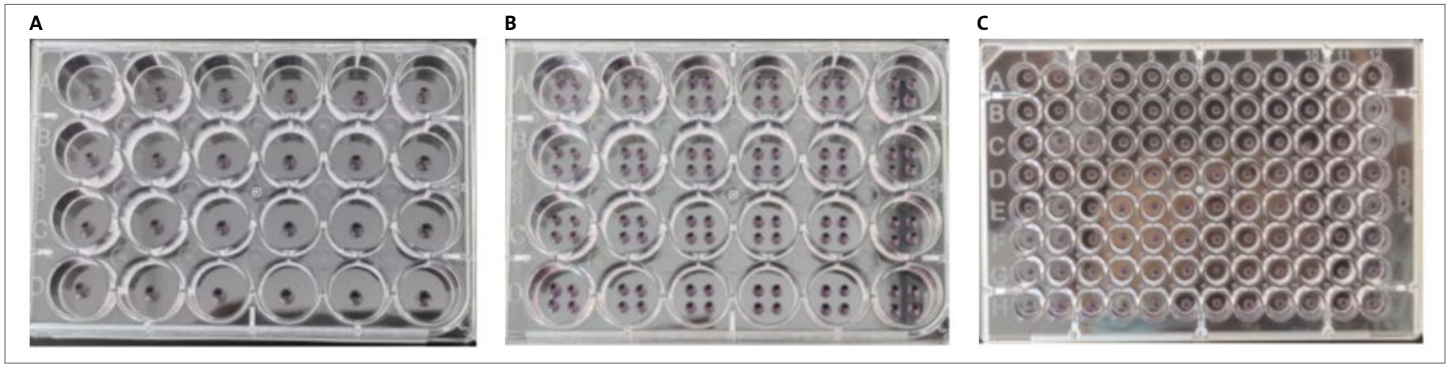

Figure 2. Uniform domes of 9 mg/mL Corning Matrigel matrix formed in each well.

Bioprinting with the Corning® Matribot® bioprinter supports spheroid and organoid formation

A549 or MDCK cells suspended in Matrigel matrix and dispensed using the Matribot bioprinter formed spheroids after 10 days of culture (Figures 3 and 4). Complex, multi-lobed mouse intestinal organoid structures were observed after 5 to 7 days of culture following passage into fragments and automatic dispensing using the Matribot bioprinter (Figure 5).

Table 2. Printing parameters for dispensing cell suspensions and mouse intestinal organoid fragments.

Printhead Parameters	
Printhead settings	Cell suspension/Corning Matrigel matrix (9 mg/mL)
Vessel selection	24-well plate
Droplet pattern per well	Three droplets
Droplet volume	10 µL
Temperature printbed	37°C
Temperature printhead	4°C
Extrusion rate	20 µL/s
Extrusion volume	12 µL
Retract volume	2 µL
Z-offset	0.3 mm
Advanced	
Extra preflow volume	0 µL
Retract rate	5 µL/s
Postflow stop time	0 s
Z-lift between wells	30 mm

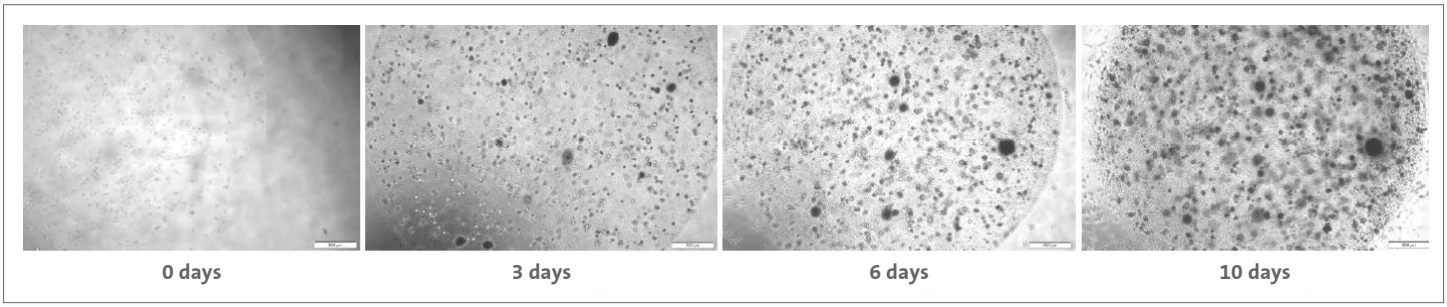

Figure 3. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports spheroid formation of A549 cells. Representative photomicrographs of A549 spheroids after 0, 3, 6, and 10 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

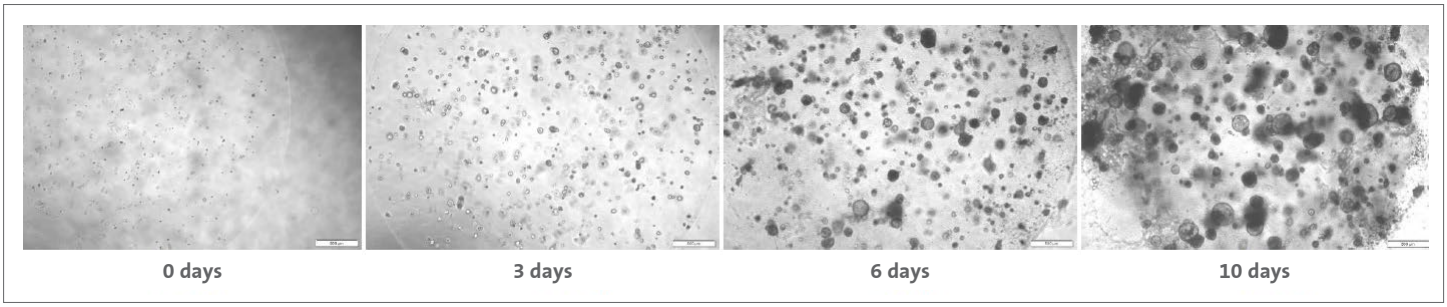

Figure 4. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports spheroid formation of MDCK cells. Representative photomicrographs of MDCK spheroids after 0, 3, 6, and 10 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

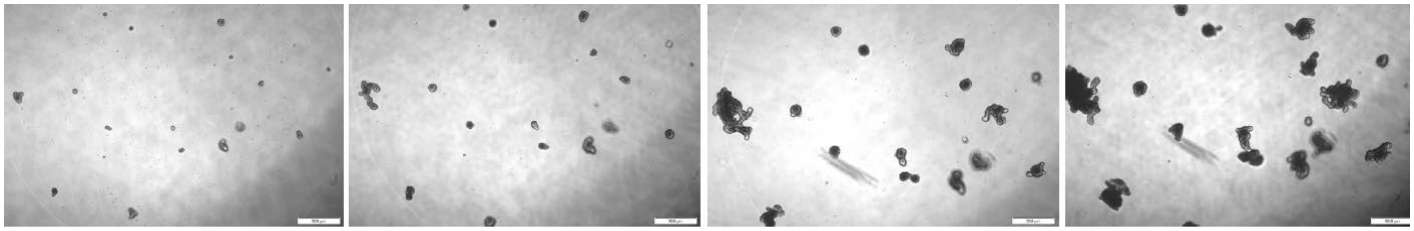

Figure 5. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports the formation of mouse intestinal organoids following passaging. Representative photomicrographs of mouse intestinal organoids after 0, 2, 5, and 7 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

Conclusions

- The Corning® Matribot® bioprinter provides a quick, high throughput platform for dispensing designated volumes of bioinks and extracellular matrices such as Corning Matrigel matrix using single droplet or multiple droplet patterns in different vessels.
- This method supports the formation of spheroid-forming cells (A549 and MDCK) from single cells and organoid-forming cells (mouse intestinal organoids).

References

1. Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. JAMA. 2006 Oct 11;296(14):1731-2.
2. Jiang T, Munguia-Lopez J, Flores-Torres S, et al. Bioprintable alginate/gelatin hydrogel 3D in vitro model systems induce cell spheroid formation. J Vis Exp. 2018 Jul 2;(137):57826.
3. Renner H, Grabos M, Becker KJ, et al. A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids. Elife. 2020 Nov 3;9: e52904.
4. Jian H, Wang M, Wang S, et al. 3D bioprinting for cell culture and tissue fabrication. Bio-des. Manuf. 2018, 1, 45-61.

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