Corning® Matribot® Bioprinter Printed Domes for Organoid Assays

Application Note

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Introduction

Patient-derived pancreatic organoids are capable of recapitulating tissue from the original tumor[1] making them an ideal model for generating biobanks, compound library testing, and personalized cancer drug screening. Most organoid assays require extracellular matrices (ECMs) such as Corning Matrigel® matrix for self-organization and differentiation. Handling ECMs can be challenging due to its temperature sensitivity and viscosity[2]. Manually dispensing such matrices in the small volumes required for screening can lead to inaccurate dispensing volumes which in turn results in inconsistent organoids cultured in each dome, leading to unreliable results. To address these challenges, an automated dispensing protocol has been developed utilizing the Corning Matribot bioprinter which is specifically designed to handle viscous and temperature-sensitive hydrogels such as Corning Matrigel matrix. Data presented here demonstrates the ability of the Corning Matribot bioprinter to accurately and consistently dispense the same number of organoids in a single 3 µL dome dispensed into the center of each well of a 96-well microplate. Using this process, up to 8 microplates were dispensed in succession from a single syringe. The resulting plates demonstrated CV values of less than 15%, without significant variability in the number of organoids dispensed into each dome. An assay was then developed to assess the toxicity of several chemotherapeutic agents traditionally used for treating pancreatic cancer, against the cultured organoids. These data demonstrate an automated way to assay patient-derived organoids cultured in Matrigel matrix domes.

Materials and Methods

Organoid Culture

Pancreatic cancer organoids HCM-CSHL-0094-C25 (ATCC® PDM-41) were cultured per vendors recommendation using Corning Matrigel matrix for organoid culture (Corning 356255) as previously described in Corning Matrigel Matrix 3D Plates for High Throughput Organoid Assays application note (CLS-AN-616). Once organoids were ready for use, Matrigel matrix domes were collected via pipetting with Axygen® Maxymum Recovery® 1000 µL tips (Corning TF-1000-L-R-S) using Advanced DMEM complete medium at 2°C to 8°C (Table 1). Organoids were transferred to an autoclaved Costar® 1.7 mL low binding microcentrifuge tube (Corning 3207) and were centrifuged at 450 x g for 5 minutes. Pelleted organoids were resuspended in 500 µL of Accutase® cell detachment solution (Corning 25-058-CI) for approximately 15 minutes at 37°C. Five hundred microliters of complete pancreatic organoid medium (Table 2) was then added to the dissociated organoids prior to enumerating.

Table 1. Advanced DMEM Complete Medium Composition.

Description	Vendor	Cat. No.	Final Concentration
Advanced DMEM with F-12 Hams	Thermo Fisher	12634	1X
Corning® glutagro™	Corning	25-015-CI	2 mM
HEPES	Corning	25-060-CI	10 mM
Penicillin/ Streptomycin	Corning	25-002-CI	1X

Table 2. Pancreatic Organoid Medium Composition.

Description	Vendor	Cat. No.	Final Concentration
Wnt-3A Conditioned Medium	–	–	50%
N-Acety-L-cysteine	MilliporeSigma	A9165-5G	1.25 mM
Noggin	Peprotech	50-399-007	100 ng/mL
R-Spondin 1	R & D Systems	46-45RS-100	250 ng/mL
B-27 Supplement	Invitrogen	17-504-044	1X
Gastrin	Sigma-Aldrich	G9145 1	0 nM
Nicotinamide	Sigma-Aldrich	N0636	10 mM
human EGF	Peprotech	AF-100-15	50 ng/mL
human FGF-10	R & D Systems	345FG025	100 ng/mL
TGF-ß type I Receptor Inhibitor	Tocris	29-395-0	500 nM
Primocin	InvivoGen	NC9392943	0.5X
Advanced DMEM Complete Medium	–	–	Remaining

Preparation for Printing

In preparation for printing, 96-well clear flat bottom microplates (Corning 3596) were placed at 37°C for at least 24 hours. Additionally, a 3 mL syringe (BD 309657) already affixed with a 22G bioprinting nozzle (Corning 6167) was placed at 2°C to 8°C until ready for filling.

Printing

Prior to preparing the pancreatic organoid ink, the Corning® Matribot® bioprinter (Corning 6150) printhead was set to 2°C to maintain printability of the ink. In the meantime, pancreatic organoids were diluted to a concentration of 200,000 cells/mL in pancreatic organoid medium containing a final concentration of 7 mg/mL Corning Matrigel® matrix for organoid culture (Corning 356255) and kept on ice. Once the temperature of the printhead achieved the setpoint, the pre-chilled syringe was filled with pancreatic organoid ink via gentle manual aspiration through the nozzle and loaded into the precooled Corning Matribot bioprinter printhead. The automatic calibration function was used to ensure each plate was properly aligned prior to single 3 µL droplets of pancreatic organoid ink were dispensed into each well of the microplate using settings shown in Table 3. After printing, plates were allowed to incubate at room temperature for 5 minutes prior to moving to 37°C for 20 minutes of polymerization of the matrix. After polymerization, 100 µL of complete media containing 10 µM Rock inhibitor (MilliporeSigma Y0503) was added to each well. Medium (minus the Rock inhibitor) was exchanged every other day.

Table 3. Microplate Settings

Parameter	Setting
Temperature printhead	2°C
Extrusion rate	60 µL/s
Extrusion volume	6.0 µL
Retract volume	3.0 µL
Droplet volume	3.0 µL
Z-offset	0.0 mm
Extra preflow volume	0.5 µL
Retract rate	60 µL/s
Postflow stop time	0.5 s
Z-lift between wells	20 mm

Assay

Pancreatic organoid domes were imaged on Day 4 via brightfield using the CellInsight™ CX7 high-content screening platform (Thermo Fisher). A single 2X objective image was taken of each well to analyze the number of objects in each dome with the supplied HSC Studio™ cell analysis software. After analysis, the medium was replaced with that containing serially diluted compounds of 5-fluorouracil (Acros 228440010), Irinotecan (Tocris 2688), Bortezomib (Selleckchem S1013), and Paclitaxel (Enzo BMLT104- 0005) starting at 0.4 µm. Organoids were cultured with either the diluted pharmaceutically active compound (PhAC) or DMSO only matched control for 3 additional days. On the final day of the assay, medium was exchanged for 50 µL of HBSS (Corning 21-023-CM) containing 10 µg/mL of Hoechst 34580 (Thermo Fisher H21486) and 10 µg/mL of propidium iodide (PI; AnaSpec 83215). Organoids were incubated with staining solution for 30 minutes at 37°C before assessing total fluorescent intensity of the PI from each well using the CellInsight CX7.

Results and Discussion

A major challenge with using patient-derived organoids for drug testing is the ability to accurately and reproducibly dispense small volumes of precious organoids. Here we demonstrated the Corning Matribot bioprinter's capability to meet these needs using patient-derived pancreatic cancer organoids. We used brightfield analysis with the CellInsight CX7 high-content screening platform to enumerate the organoids and demonstrated the quality and consistency of Corning Matrigel matrix dispensing. Figure 1 shows a representative scan of an entire 96-well microplate of pancreatic cancer organoids with one well digitally zoomed in. The images are shown with and without masks used for subsequent analysis. Figure 2 demonstrates the ability to dispense the same number of organoids in each well of a 96-well microplate while Figure 3 highlights the low well-to-well variation with coefficients of variation of less than 15%.

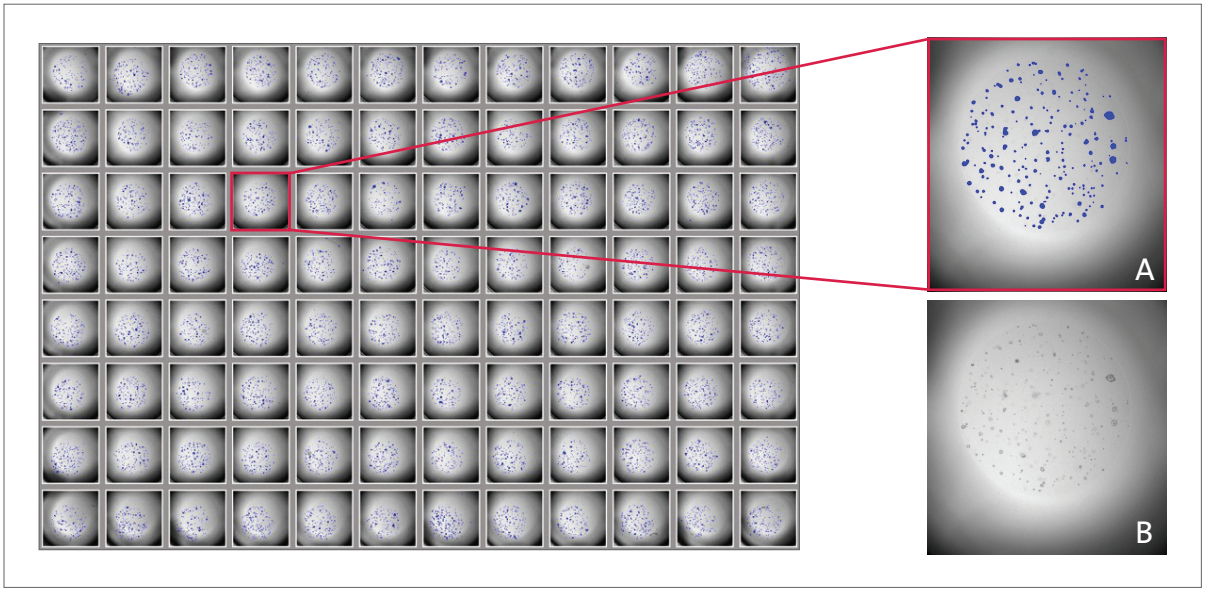

Figure 1. Organoid image analysis. Representative scan of entire 96-well microplate of pancreatic cancer organoids domes with one well digitally zoomed in with (A) and without (B) the mask used for analysis.

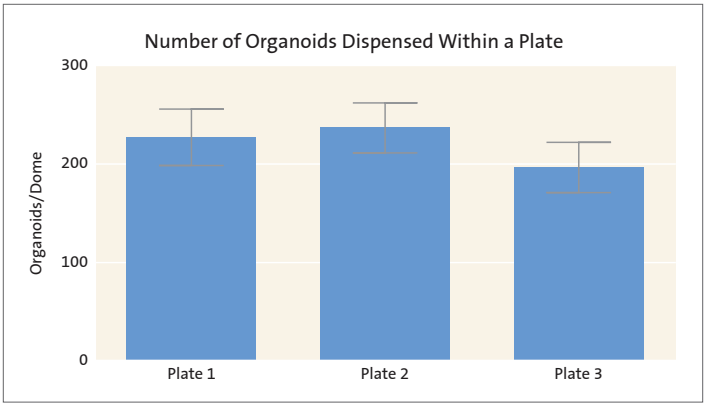

Figure 2. Average organoids dispensed per plate. Average number of pancreatic cancer organoids dispensed in each 96-well microplate from 3 independent studies.

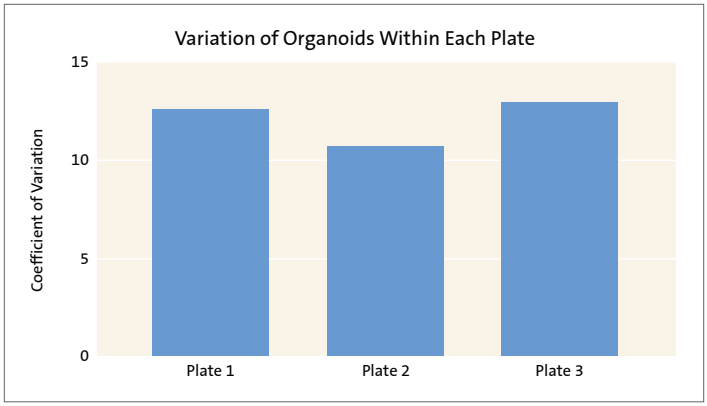

Figure 3. Coefficient of variation. Coefficient of variation (CV) in the number of patient-derived pancreatic cancer organoids in each well across a 96-well microplate from 3 independent studies.

To illustrate the ability of the bioprinter to fill multiple microplates from a single syringe, we were able to dispense into 8 microplates, in succession, without removing or mixing the syringe. Due to the small cluster size of the pancreatic cancer organoids and viscosity of the Matrigel matrix, we saw little variability in the number of organoids dispensed from plate-to-plate (Figure 4). A Dunnett's post hoc test found no statistical difference in the number of organoids dispensed between the first microplate and any other microplate ($p > 0.05$).

As a proof of concept for the use of organoids for drug testing, the pancreatic cancer organoids were exposed to serially diluted PhAC for 3 days. After PhAC exposure, pancreatic cancer organoids were stained with Hoechst and PI to assess viability. Representative images of organoids exposed to 0.4 µM Paclitaxel, Bortezomib or DMSO matched control can be seen (Figure 5). Average dose-dependent responses to 5-Fluorouracil, Irinotecan, Bortezomib and Paclitaxel were analyzed via high content analysis (Figure 6) resulting in TC50s of 0.0052, 0.0108, 0.0044, and 0.0008 µM, respectively.

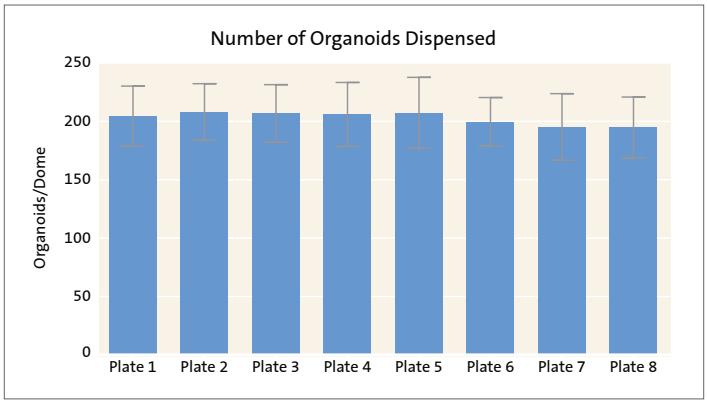

Figure 4. Organoids dispensed from a single syringe. Average number of patient-derived pancreatic cancer organoids in each well across a 96-well microplate dispensed from a single syringe. $P > 0.05$ using Dunnett's post-test to compare plate 1 to all other plates.

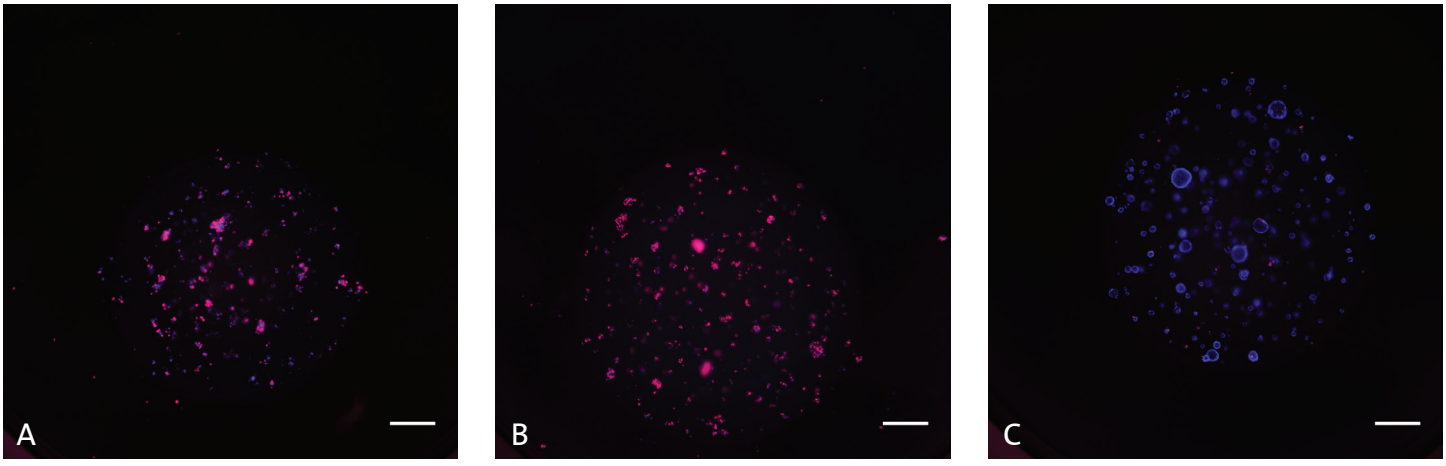

Figure 5. Representative organoid viability after drug exposure. Representative photomicrographs of pancreatic cancer organoids after 3 days of exposure to drugs or DMSO control. Images staining with Hoechst 34580 and propidium iodide. (A) 0.4 µM Paxlitaxel, (B) 0.4 µM Bortezomib, and (C) DMSO control. Scale is 500 µM.

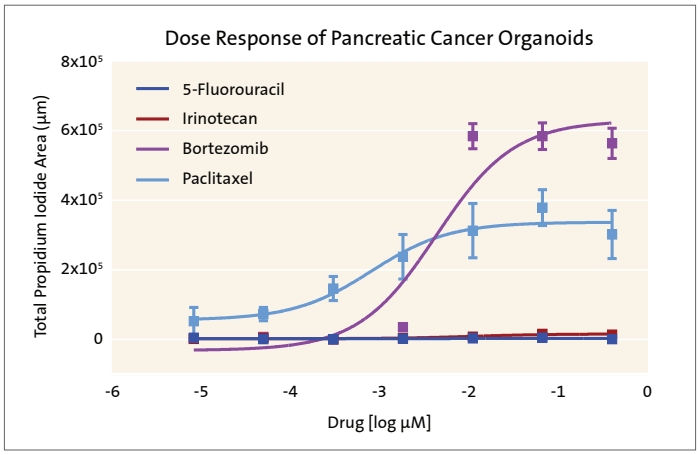

Figure 6. Dose response curves from drug exposure. Cytotoxic response of pancreatic cancer organoids after exposure to 5-Fluorouracil, Irinotecan, Bortezomib, and Paclitaxel via PI staining. Data is shown from 3 independent studies with standard error resulting in TC50s of 0.0052, 0.0108, 0.0044, and 0.0008 µM, respectively.

Conclusions

The Corning® Matribot® bioprinter is specially designed to print viscous and temperaturesensitive materials such as Corning Matrigel® matrix. The temperature-controlled printhead allows for maintaining printability of temperature-sensitive inks until polymerization is desired, making this an ideal tool for accurately and precisely dispensing cells requiring ECMs such as patient-derived organoids.

NOTE: Should you intend to use the HUB Organoid Technology methods for commercial purposes, please contact HUB at info@huborganoids.nl for a commercial use license.

References

1. Romero-Calvo I, et al. Human Organoids Share Structural and Genetic Features with Primary Pancreatic Adenocarcinoma Tumors PDAC Organoids Mimic Patient Disease. Mol Cancer Res 17.1 (2019): 70-83.
2. Co, JY, et al. Controlling the Polarity of Human Gastrointestinal Organoids to Investigate Epithelial Biology and Infectious Diseases. Nature protocols 16.11 (2021): 5171-5192.

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