Corning® Matrigel® Matrix Supplemented Media for Pancreatic Cancer Organoid Assays

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Application Note

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Introduction

It has been proven that organoids better recapitulate the complexity and population diversity of patients as compared to more traditionally used cell lines thus resulting in the need to develop organoid models for higher throughput and more predictive drug discovery campaigns[1]. One major hurdle to automating these models is the manual method used to culture organoids which often consists of maintaining organoids in domes of extracellular matrices (ECMs)[2]. These ECMs are viscous and temperature-sensitive which can present challenges when used with automated liquid handling systems. Here, we demonstrate an alternative technique for maintaining organoids in Corning Elplasia® microplates using media supplemented with Corning Matrigel matrix[3]. This method allows for lower concentrations of Matrigel matrix in the media, making automation easier. Additionally, the Elplasia microplate, with its microcavity substrate, allows for significantly more data points per well since organoids are better separated and are in a more uniform focal plane than with dome cultures. The combination of the Elplasia microplate and Matrigel matrix supplementation allows for easier image analysis especially when used in conjunction with the Keyence BZ-X800 all-in-one fluorescence microscope. To evaluate this new technique, propidium iodide (PI) and nuclear staining were used to quantify organoid viability after drug exposure.

Materials and Methods

Organoid Culture

Pancreatic cancer organoids HCM-CSHL-0094-C25 (ATCC® PDM-41™) were cultured per vendor's recommendation using Corning Matrigel matrix for organoid culture (Corning 356255) as previously described in Corning Application Note CLS-AN-616 with the replacement of PancreaCult™ Organoid media (STEMCELL Technologies 100-0781) for in-house made media. 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 20 minutes at 37°C with gentle pipetting to dissociate organoids into single cells. Five hundred microliters of PancreaCult Organoid media were 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

Elplasia Microplate Seeding

In preparation for seeding, 96-well Elplasia microplates (Corning 4442) were pre-wet to remove trapped air by adding 50 µL per well of media containing 10 µM ROCK inhibitor (MilliporeSigma Y0503) and centrifuged at 500 x g for 1 minute. Cells were centrifuged at 300 x g for 4 minutes and resuspended to 60,000 cells/mL in PancreaCult Organoid media containing 0.2 mg/mL of Matrigel matrix for organoid culture and 10 µM rock inhibitor. 150 µL of cell suspension were added to each well of a 96-well Elplasia microplate.

Immunocytochemistry Staining

Organoids were fixed by aspirating medium and replacing with 200 µL of 4% paraformaldehyde (PFA) (Boston BioProducts BM-155) per well and incubated for 1 hour at room temperature. PFA was removed and washed with 200 µL of phosphate buffered saline (PBS; Corning 21-030-CM) after which the organoids were permeabilized with 0.2% Triton X (Integra Chemical Company T756.30.30) for 5 minutes and then blocked with 5% donkey serum (Equitech-Bio, Inc. SD-2676) in PBS for 1 hour. Finally, organoids were stained with 50 µL per well of 10 µg/mL PDX-1 (R&D Systems IC2419T) and 50 µg/mL CK-19 (Proteintech CL488-10712) for 2 hours. Organoids were imaged using the BZ-X800 all-in-one fluorescence microscope.

Assay

Seventy-two hours after seeding pancreatic cancer organoids, 10 µL media containing serial dilutions of bortezomib (Tocris 7282), MG-132 (Tocris 1748), paclitaxel (Enzo BML-T104-0005) or matched DMSO containing media control were added to each well. After 24 hours of drug exposure, 10 µL of PBS containing 340 µg/mL Hoechst 34580 (Thermo Fisher H21486) and 136 µg/mL PI (AnaSpec 83215) were added to each well. Plates were incubated at 37°C for 1 to 2 hours until complete staining was observed. Images were captured with the BZ-X800 all-in-one fluorescence microscope using a 2X objective.

Results and Discussion

A major constraint in automating organoid workflows into drug discovery campaigns is the challenge of dispensing viscous and temperature-sensitive ECMs that are typically needed for organoid culture. Recently, there have been publications demonstrating the ability to culture patient-derived organoids in media that is supplemented with ECMs instead of fully embedding cultures. These protocols greatly simplify organoid workflows by reducing ECM handling steps and the concentrations required. Here we demonstrated this technique by combining Corning's Ultra-Low Attachment (ULA) surface coated Elplasia microplates with media supplemented with Matrigel matrix for organoid culture. The low concentration of Matrigel matrix in combination with the Elplasia microplate allowed cells to settle evenly across the microcavities and expand with time (Figure 1). Most importantly, pancreatic cancer organoids demonstrated appropriate morphology and were positive for gastroenteropancreatic and hepatobiliary epithelial marker CK-19 and pancreatic duct lineage marker PDX-1 (Figure 2).

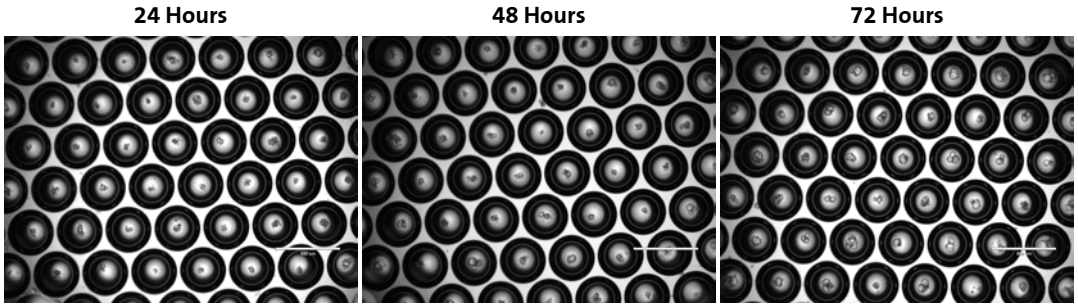

Figure 1. Pancreatic cancer organoids in a Corning Elplasia microplate. Pancreatic cancer organoids in Elplasia microplates 24, 48, and 72 hours after seeding. Images taken using a 2X objective. Scale bar is 800 µm.

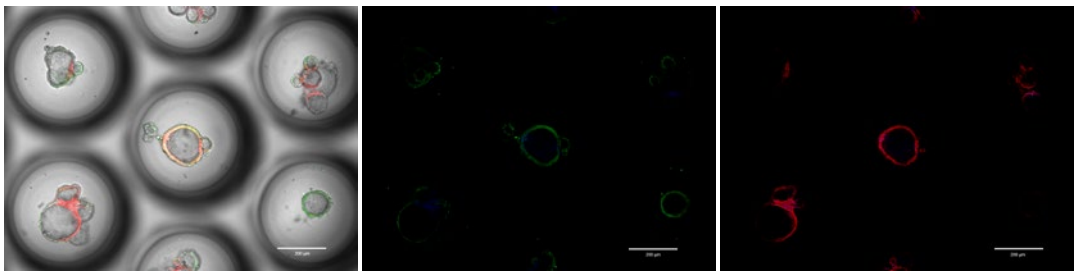

Figure 2. Stained pancreatic cancer organoids. Pancreatic cancer organoids stained with CK-19 (green) and PDX-1 (red) imaged on the Keyence BZ-X800 all-in-one fluorescence microscope. Scale is 200 µm.

To demonstrate the ability to use Ultra-Low Attachment surface coated Elplasia microplates with media supplemented with Matrigel matrix for organoid culture, we treated pancreatic cancer organoids with serially diluted concentrations of drugs for 24 hours. After drug exposure, pancreatic cancer organoids were stained with Hoechst and PI to assess viability. Representative images of organoids exposed for 24 hours to 0.5 µM bortezomib, MG-132, paclitaxel or DMSO matched control were easily obtained using the BZ-X800 all-in-one fluorescence microscope (Figure 3). It can also be seen that even after several days of culture with drug

and staining additions, organoids remain largely within their original microcavities. Average dose-dependent responses to bortezomib, MG-132, paclitaxel were analyzed via the Keyence BZX Analyzer (Figure 4) resulting in TC_{50} values of 0.0032, 0.063, 0.00138 µM, respectively.

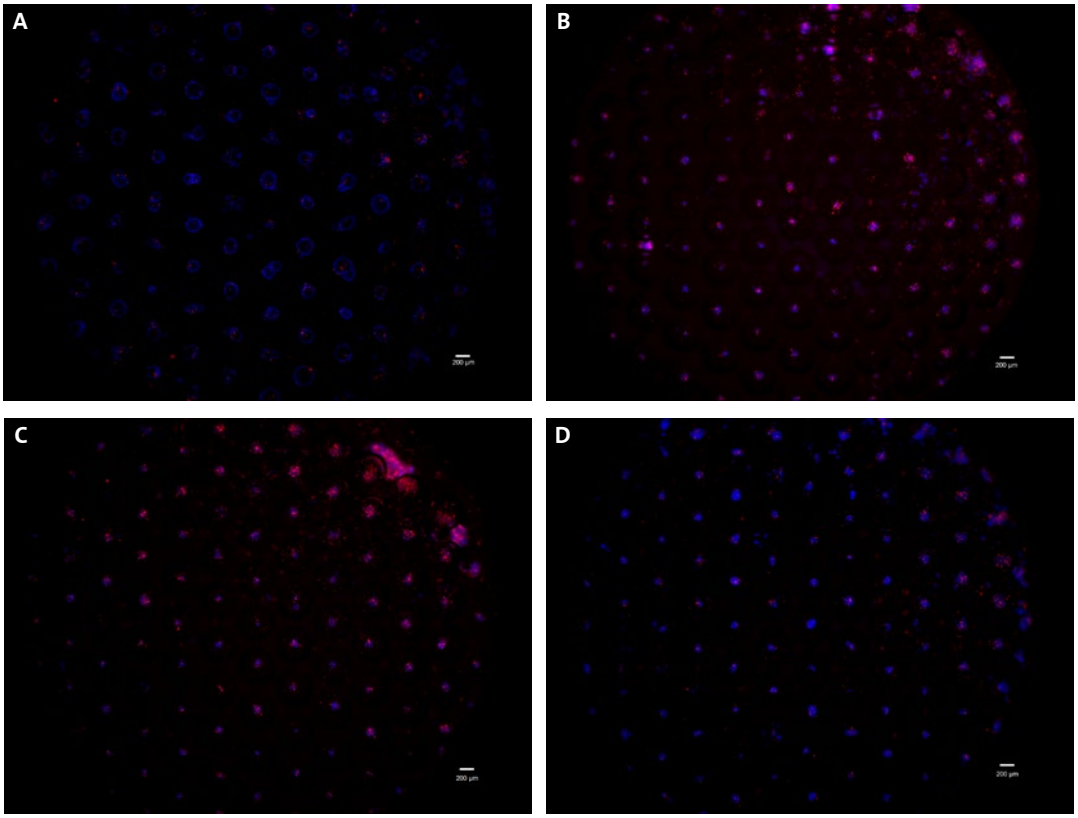

Figure 3. Pancreatic cancer organoids After exposure to drugs or DMSO Control Representative photomicrographs of pancreatic cancer organoids stained with PI and Hoechst 24 hours after exposure to DMSO control (A), 0.5 µM Bortezomib (B), 0.5 µM MG-132 (C), and 0.5 µM Paclitaxel (D). Scale bar 200 µm.

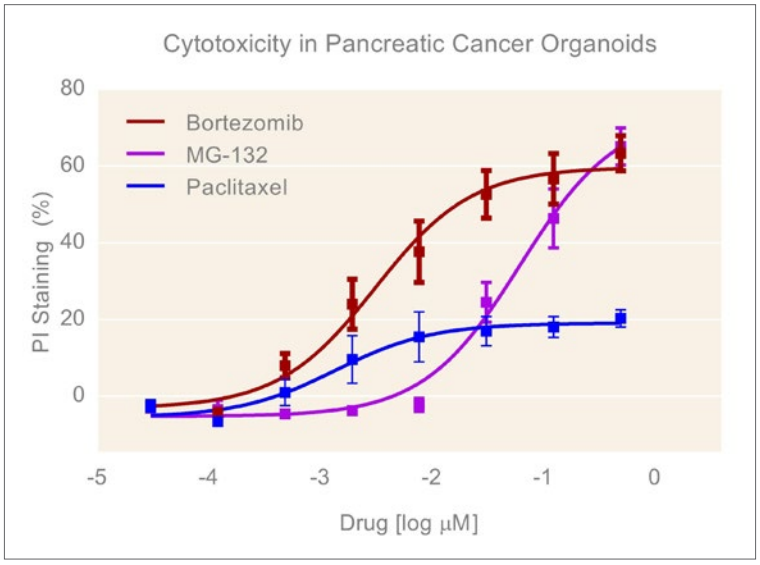

Figure 4. Cytotoxicity in pancreatic cancer organoids. Average dose-dependent responses of pancreatic cancer organoids, 24 hours after exposure, to serially diluted drugs. Data is average from 3 independent studies shown with standard deviation, n = 9 wells. TC_{50} MG-132 = 0.063 µM, Bortezomib = 0.0032 µM, and Paclitaxel = 0.001382 µM.

Conclusions

The combination of Corning® Elplasia® microplates and media supplemented with Corning Matrigel® matrix for organoid culture allowed for easier automated workflows for drug discovery campaigns. Using this technique reduced challenges associated with automated handling of viscous and temperature-sensitive ECMs resulting in organoids that were more evenly distributed and easier to image. Importantly, Pancreatic cancer organoids cultured with media supplemented with 0.2 mg/mL Matrigel matrix maintained typical morphology and expressed CK-19 and PDX-1 markers.

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

References

1. Shree B, Clevers H, and Shen X. Promises and challenges of organoid-guided precision medicine. Med 2.9 (2021): 1011-1026.
2. Baker LA, Tiriac H, and Tuveson DA. Generation and culture of human pancreatic ductal adenocarcinoma organoids from resected tumor specimens. Pancreatic Cancer: Methods and Protocols (2019): 97-115.
3. Tan Tao, et al. Low-viscosity matrix suspension culture for human colorectal epithelial organoids and tumoroids. Bio-protocol 12.8 (2022): e4394-e4394.

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