Corning® Matrigel® Matrix 3D Plates for High throughput Organoid Assays

Application Note

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Introduction

The use of organoids as research tools has become more common due to their ability to better recapitulate disease as compared to more traditional models[1]. Additionally, organoids show great promise in personalized medicine, as biopsies can be used to generate organoids that maintain many functional and genomic characteristics of the donor patient[2]. In order to use organoids as a model, it is essential to maintain proper morphology and polarity. In the body, basement membrane is responsible for helping cells to establish and maintain polarity[3]. This process can be modeled and assayed *in vitro* by culture of epithelial cells in an extracellular matrix. Corning Matrigel matrix has been demonstrated to enable polarized epithelial structures to form *in vitro* and can be used to create and maintain a wide variety of organoid models[2]. To increase the throughput for screening with 3D models, Corning has developed 96- and 384-well microplates pre-dispensed with Matrigel matrix specifically for 3D applications. The current study highlights the use of pre-coated Matrigel matrix-3D plates to screen pancreatic cancer organoids.

Materials and Methods

Organoid Culture

Pancreatic cancer organoids HCM-CSHL-0094-C25 (ATCC® Cat. No. PDM-41™) were cultured as recommended by the vendor. In brief, organoids were resuspended in Corning Matrigel matrix for organoid culture (Corning Cat No. 356255) and AdDF complete medium (Table 1) at 60% Matrigel matrix:cell volume. 24-well plates (Corning Cat. No. 3524) were incubated at 37°C in a humidified incubator for a minimum of 24 hours prior to use. Five to seven microliter domes of pancreatic cancer organoids were placed in several wells of a 24-well multiwell plate using pre-chilled Axygen® Maxymum Recovery® 200 µL tips (Corning Cat. No. T-200-C-L-R-S). Once domes were plated, plates were inverted in the laminar flow hood for 5 minutes. Plates were then transferred to a 37°C incubator for another 15 minutes in the inverted position. Once domes had fully polymerized, 500 µL of pancreatic organoid medium (Table 2) with a final concentration of 10 µM Rock inhibitor (MilliporeSigma Cat. No. Y0503) was added to each well. Medium was changed every 2 to 3 days for fresh pancreatic organoid medium without Rock inhibitor. When organoids were ready for passage, domes were collected by pipetting with Axygen Maxymum Recovery 1000 µL tips (Corning Cat. No. T-1000-C-L-R-S). Organoids were resuspended in 2°C to 8°C AdDF complete and centrifuged at 450 x g for 5 minutes. Precipitated organoids were resuspended in 500 µL of AdDF complete and transferred to autoclaved Costar® 1.7 mL low binding microcentrifuge tubes (Corning Cat. No. 3207). Organoids were sheared by triturating with a 20-gauge blunt needle (SAI Infusion Technologies Cat. No. B20-100) attached to a 1 mL syringe (Fisher Scientific Cat. No. 14-955-456). Sheared organoids were centrifuged at 90 x g for 5 minutes. Organoids were then resuspended in AdDF complete with 60% Matrigel matrix volume at a dilution between 1:4 and 1:8. Organoids were re-plated as previously described until ready for assay set up.

Table 1. AdF Complete Medium

Description	Vendor	Cat. No.	Final Concentration
Advanced DMEM/F-12 (Dulbecco's Modified Eagle Medium/Ham's F-12)	Thermo Fisher	12634	1X
Corning glutagro™ solution	Corning	25-015-CI	2 mM
HEPES buffer solution	Corning	25-060-CI	10 mM
Penicillin-Streptomycin solution	Corning	30-002-CI	1X

Table 2. Pancreatic Organoid Medium

Description	Vendor	Cat. No.	Final Concentration
WNT3a Conditioned medium			50%
N-Acetylcysteine	Sigma-Aldrich	A9165-5G	1.25 mM
Noggin	Peprotech	50-399-007	100 ng/mL
Rspondin-1	R&D Systems	46-45RS-100	250 ng/mL
B27 Supplement	Invitrogen	17-504-044	1X
Gastrin	Sigma-Aldrich	G9145	10 nM
Nicotinamide	Sigma-Aldrich	N0636	10 mM
hEGF	Peprotech	AF-100-15	50 ng/mL
FGF-10	R&D Systems	345FG025	100 ng/mL
TGFb type I Receptor Inhibitor	Tocris	29-395-0	500 nM
Primocin	Invivogen	NC9392943	0.5X
AdDF+++			Remaining

Assay Set Up

The day prior to assay set up, Corning® Matrigel® matrix-3D plates (Corning Cat. No. 356257) were placed at 4°C to thaw overnight. On the day of seed, Corning Matrigel matrix-3D plates were placed at 37°C to polymerize for at least 1 hour. While polymerizing, organoids were collected and centrifuged as previously described. Organoids were dissociated to single cells by incubating with Accutase® (Corning Cat. No. 25-058-CI) for approximately 15 minutes with gentle pipetting. Pancreatic cancer organoids were diluted to 50,000 cells/mL in pancreatic organoid medium containing 10 µM Rock and 0.09 mg/mL Matrigel matrix for organoid culture. Twenty microliters of pancreatic cancer organoid cell suspension were added to each well of the polymerized Corning Matrigel matrix-3D plate. Organoids were incubated for 48 hours prior to treating with drugs.

Screen

After 48 hours, 20 µL of 20 µM compounds from the Enzo Cancer Library (Enzo Cat. No. ENZ-LIB102), 20 µM paclitaxel (Enzo Cat. No. BML-T104-0005), or media control with matched DMSO concentration were added to the wells. Pancreatic cancer organoids were cultured with compounds for 5 days. On day 5, 40 µL of CellTiter-Glo® 3D (Promega Cat. No. G9683) was added to each well. Plates were shaken for 5 minutes and then incubated at room temperature for another 25 minutes prior to reading on PerkinElmer EnVision® Multimode Plate Reader.

Follow Up Doses

After 48 hours of culture on Corning Matrigel matrix-3D plates, 20 µL of paclitaxel, daunorubicin (MilliporeSigma Cat. No. D8809), gemcitabine (MilliporeSigma Cat. No. G6423), 5-fluorouracil (Acros Cat. No. 228440010), oxaliplatin (AdipoGen Cat. No. AG-CRI-3592-M005), bortezomib (Selleckchem Cat. No. S1013), or media matched with DMSO were added to pancreatic cancer organoids. Pancreatic cancer organoids were cultured for 5 additional days and assayed with CellTiter-Glo 3D, as previously described.

Results and Discussion

For high throughput assays to provide referenceable data, it is necessary to reduce or control as many variables as possible. For organoid assays, one of the major contributors to variability is the extracellular matrix coating process. Extracellular matrices tend to be temperature-sensitive and viscous which can make uniform coating problematic. Figure 1 demonstrates typical pancreatic cancer organoid morphology after 2 days of culture on Corning Matrigel matrix-3D plates. After 2 days, drugs were added, and organoids were cultured for an additional 5 days. Figure 2 shows representative images of organoids after culture with 10 µM paclitaxel or media with DMSO control. Z' was calculated to determine the robustness of the assay. Z' greater than 0.5 were achieved in all 3 independent studies (Figure 3). Enzo Life Sciences SCREEN-WELL® Cancer Library was used to determine compound cytotoxicity for the specific organoid model being tested. The resulting cytotoxicity from 3 studies was averaged and sorted by effect. The results are shown in Figure 4. Six compounds were selected for follow up of dosing either because they were a hit in the screen or because they are traditionally provided as treatment for pancreatic cancer. Figure 5 shows varying degrees of sensitivity between the compounds. Gemcitabine and paclitaxel are often standard recommended treatments for pancreatic cancer[4]. Our study found these compounds to be highly effective at inducing cytotoxicity with the pancreatic cancer organoids tested. (EC_{50} values of 0.5139 nM for paclitaxel and 3.887 nM for gemcitabine). Other traditional chemotherapeutics offered for pancreatic cancer, 5-fluorouracil and oxaliplatin, have been shown to be less effective for pancreatic cancer which corresponded with our data[5,6]. We also found Bortezomib, a proteasome inhibitor that has shown promise as a therapeutic for pancreatic cancer, to be highly effective[7].

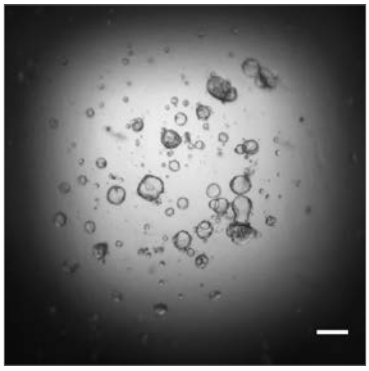

Figure 1. Typical pancreatic cancer organoid morphology. Representative image of typical pancreatic cancer organoid morphology after two days cultured on Corning® Matrigel® matrix-3D plates. Scale bar 200 µm.

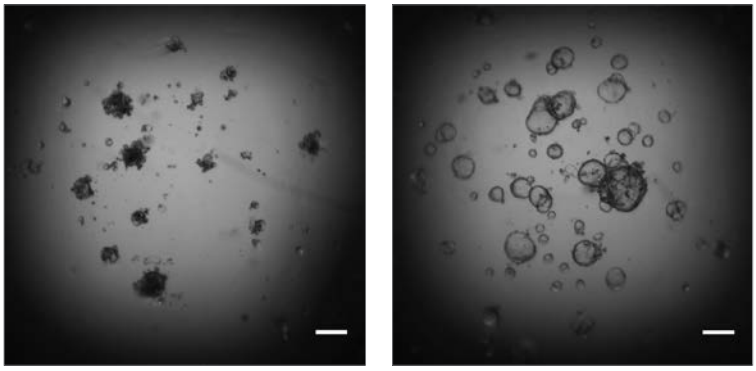

Figure 2. Typical morphology of organoids after exposure to paclitaxel or media control. Representative image of pancreatic cancer organoids after five days of exposure to 10 µM paclitaxel (left) or media with matched DMSO (right). Scale bar 200 µm.

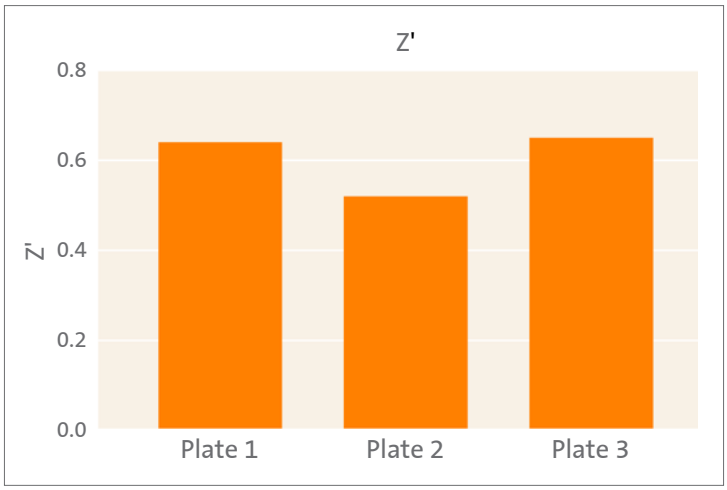

Figure 3. Assay is robust. Z' calculated from comparing viability of pancreatic cancer organoids exposed to paclitaxel or media control. All values were above 0.5 indicating a robust assay.

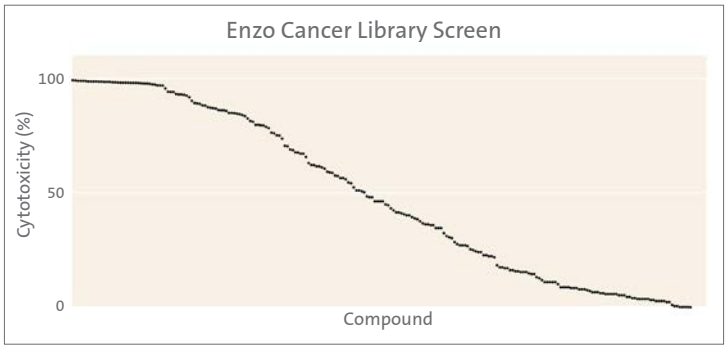

Figure 4. Enzo cancer library screen. Average percent reduction in pancreatic cancer organoid viability compared to media with DMSO response. Data is average of 3 independent screens and sorted by effect on viability.

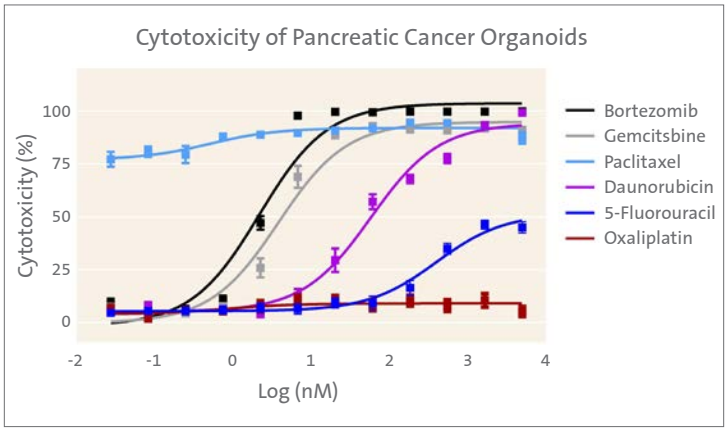

Figure 5. Follow up doses. Follow up cytotoxicity of selected compounds from library. Data is shown as average from 3 independent studies with standard error bars. N = 12 for each dose.

Conclusions

For organoids to become commonplace in personalized medicine or drug discovery, it is essential that the methods used become amenable to high throughput. Corning® Matrigel® matrix-3D plates eliminate one of the typical challenges encountered when self-coating microplates with extracellular matrices (i.e., Matrigel matrix) by providing a ready-to-use option. Corning Matrigel matrix-3D plates provide the convenience and consistency required for 3D high throughput applications such as organoid screening. Our results demonstrated how Corning Matrigel matrix-3D plates can be used to screen pancreatic cancer organoids in order to identify ideal chemotherapeutic treatments.

NOTE: Should you intend to use the HUB Organoid Technology methods for commercial purposes, please contact HUB at info@hub4organoids.nl for a commercial use license.

References

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