Culturing Human Intestinal Organoids with Corning® Matrigel® Matrix for Organoid Culture

Application Note

CORNING

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Introduction

During the last decade, the number of research articles on organoids has steadily increased[1]. This trend can be attributed to properties of organoids, such as their similarity to primary tissue, genomic stability, and that they are comprised from parallel cell types to the original tissue[2]. Although a great tool for research, cultured organoids can be inconsistent and divergent during growth and maintenance due to inconsistency in sample sourcing and reagents used[3]. Corning offers an extracellular matrix (ECM) specifically for culturing organoids to lessen potential variability. Each lot of Corning Matrigel matrix for organoid culture is tested for elastic modulus and ability to form a stable dome; both of which are important for organoid culture[4]. In this article, we demonstrate use of Corning Matrigel matrix for organoid culture to maintain human intestinal organoids (HIO) for multiple passages.

Materials and Methods

Human intestinal organoids with a cystic fibrosis phenotype were purchased from Hubrecht Organoid Technology (HUB; Utrecht, Netherlands). Organoids were cultured per HUB methodologies[5]. In brief, organoids were resuspended in Corning Matrigel matrix for organoid culture (Corning 356255) and AdDF complete medium (Table 1) at 55% to 65% Matrigel: cell volume. At least 24 hours prior to plating HIO, 24-well plates (Corning 3524) were incubated at 37°C in a humidified incubator. Four to five domes of 5 to 7 µL of HIO were placed in several wells of a 24-well multiple well plate using pre-chilled Axygen® 200 µL Maxymum Recovery® tips (Corning T-200-C-L-R-S). Once domes were plated, plates were inverted in the laminar flow hood for 5 minutes. Inversion of the plates allows for organoids to settle away from the plate surface where they might attach and differentiate. 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 colon medium (Table 2) with a final concentration of 10 µM Rock inhibitor (MilliporeSigma Y0503) was added to each well. Medium was changed every 2 to 3 days for fresh colon medium without Rock inhibitor. When organoids were ready for passage, domes were collected by pipetting with Axygen 1000 µL Maxymum Recovery tips (Corning 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. Pelleted HIO were resuspended in 500 µL of AdDF complete and transferred to autoclaved Costar® 1.7 mL low binding microcentrifuge tubes (Corning 3207). HIO were sheared by triturating with a 20-gauge blunt needle (SAI Infusion Technologies B20-100) attached to a 1 mL syringe (Fisher Scientific 14-955-456).

Table 1. AdDF complete Medium

Item	Vendor	Cat. No.	Final Concentration
Advanced DMEM with F-12 Hams	Thermo Fisher	12634010	1X
Corning glutagro™	Corning	25-015-CI	2 mM
Hepes	Corning	25-060-CI	10 mM
Penicillin/Streptomycin	Corning	30-002-CI	1X

Table 2. Colon Medium

Item	Vendor	Cat. No.	Final Concentration
WNT3a Conditioned Medium			50%
N-Acetylcysteine	MilliporeSigma	A9165-5G	1.25 mM
Noggin	Peprotech	50-399-007	100 ng/mL
Rspondin-3	R&D Systems	3500RS025CF	250 ng/mL
B27 supplement	Invitrogen	17-504-044	1X
Gastrin	MilliporeSigma	G9145	5 nM
Nicotinamide	MilliporeSigma	N0636	10 mM
hEGF	Peprotech	AF-100-15	50 ng/mL
p38 MAPK inhibitor (p38i) SB202190	MilliporeSigma	S7067	10 µM
TGFb type I Receptor inhibitor	Tocris	29-395-0	500 nM
Primocin	Invivogen	NC9392943	0.5X
AdDF complete Medium			Remaining

Once organoids were sheared to the desired size, the larger non-sheared organoids were allowed to settle to the bottom of the tube while the smaller organoids were transferred to a new tube. Sheared HIO were centrifuged at 90 x g for 5 minutes. Organoids were then resuspended in AdDF complete with 55-65% Matrigel matrix volume at a dilution between 1:4 and 1:6. Organoids were re-plated and cultured as previously described until ready for passage.

Representative images were taken from 3 different lots of Matrigel matrix for organoid culture preceding each passage. Prior to the fourth passage, organoids were collected for histology with Axygen 1000 µL Maxymum Recovery tips. Organoids were washed twice with 1 mL of 2°C to 8°C phosphate buffered saline (PBS; Corning 21-040-CM) per microcentrifuge tube.

After aspirating PBS, 1 mL of 2°C to 8°C Corning® Cell Recovery solution (Corning 354253) was added to each tube. Organoids were incubated at 2°C to 8°C for 5 minutes. HIO were washed with cold PBS twice more. Once all Matrigel matrix was removed, organoids were fixed with 4% paraformaldehyde for 1 hour on a shaker at room temperature. After fixation, organoids were washed two additional times with PBS prior to being given to the University of New England for histology. HIO were processed per Spheroid Processing and Embedding for Histology Guidelines for Use (Corning Lit. Code CLS-AN-043) with markers listed in Table 3.

Results and Discussion

HIO were passaged into 3 lots of Matrigel matrix for organoid culture and then subsequently passaged 3 additional times in each Matrigel matrix lot. Throughout the entire study, HIO demonstrated typical budded morphology of those derived from a donor with cystic fibrosis (Figure 1). Upon completion of the 3-passage study, HIO were fixed and sectioned to confirm the presence of appropriate intestinal cells. To assess their similarity to primary tissue, HIO from the large intestine should contain goblet cells, Paneth cells, and enterocytes[6]. Figure 2 confirms positive staining of all three cell types from the organoids cultured in three lots of Matrigel matrix for organoid culture (Corning 356255).

Conclusions

Culturing and maintenance of human organoids is complex. One of the greatest challenges is reproducibility, Corning Matrigel matrix for organoid culture aims to reduce some of this inherent variability by offering an ECM for culturing organoids. This article demonstrates that we were able to maintain similar HIO cultures from 3 different lots of Matrigel matrix for organoid culture.

Acknowledgments

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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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Table 3. Cell Specific Markers

Antibody	Vendor	Cat. No.	Concentration
Villin	Abcam	ab130751	1:400
MUC2	Abcam	ab11197	1:1000
Lysozyme	Abcam	ab108508	1:500

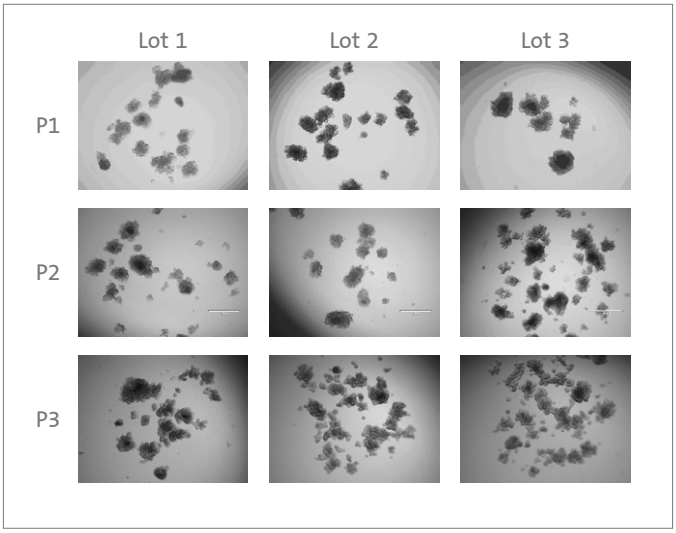

Figure 1. Typical budded morphology of CF HIOs.

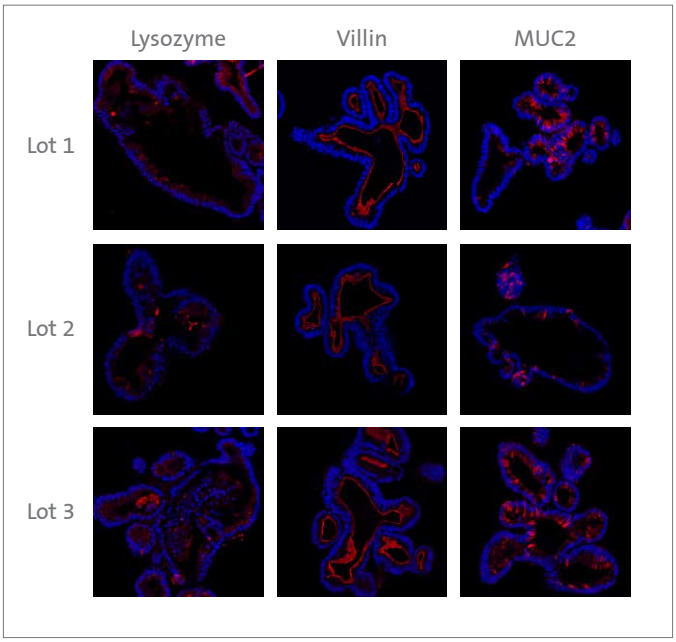

Figure 2. Positive staining or organ-specific cell types.

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