

Culture of Mouse Intestinal Organoids in Corning® Matrigel® Matrix for Organoid Culture

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Application Note

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Introduction

Organoids are defined as complex three-dimensional (3D) structures that can mimic functionality of the *in vivo* organ counterparts.^{1,2} They can be formed by the expansion and subsequent self-organization of stem cells and progenitor cells and their differentiated offspring. Organoids recapitulate numerous interactions such as cell-cell, cell-matrix, and tissue-specific physiological functions.³ They have a wide range of applications in the fields of basic biology, disease modeling, drug discovery, personalized medicine, and regenerative medicine.

In adult mammals, the intestinal epithelium is a rapidly renewing tissue and is controlled by intestinal stem cell maintenance and self-renewal.⁴ In the small intestine, self-renewing stem cells reside at the bottom of crypts and differentiate into different cell types such as enterocytes, goblet cells, paneth cells, and enteroendocrine cells. After studying the growth requirements for intestinal epithelium *in vivo*, a robust culture system has been described wherein stereotypical structures resembling the intestinal crypts (i.e., organoids) were formed and maintained in long-term culture.⁵ This culture method involved the use of basement membrane extract that mimics the Laminin-rich crypt base, as well as the addition of factors such as Wnt-agonist R-spondin-1, epidermal growth factor (EGF), and Noggin.

Corning Matrigel matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins including Laminin (a major component), Collagen IV, heparan sulfate proteoglycan, entactin/nidogen, and several growth factors that are found in normal EHS tumors.^{6,7} Corning Matrigel matrix has been used in a wide range of applications such as stem cell culture, cell attachment and differentiation, angiogenesis assays, tumor growth, and tissue engineering.^{8,9} We present Corning Matrigel matrix for organoid culture, an optimal extracellular matrix that supports organoid growth and differentiation while saving reagents and time used for lot testing. Each lot of Corning Matrigel matrix for organoid culture has a specific elastic modulus value indicating the stiffness of the gel formed and is qualified to form stable 3D domes commonly used in organoid culture protocols.

Using HUB Organoid Technology methods, we demonstrated that Corning Matrigel matrix for organoid culture supported the culture of mouse intestinal organoids (MIO) for more than 7 passages in serum-free IntestiCult™ organoid growth medium (mouse). The MIOs cultured in Corning Matrigel matrix for organoid culture domes showed a typical budding morphology and contained differentiated intestinal cells that expressed specific surface markers.

Materials and Methods

Corning Matrigel Matrix Handling

Corning Matrigel matrix for organoid culture (Corning Cat. No. 356255) was thawed by submerging a vial in an insulated bucket of ice and kept in the refrigerator (2°C to 8°C) overnight according to Guidelines for Use. Corning Matrigel matrix for organoid culture was aliquoted and stored at -80°C for long-term storage. Aliquots of Corning Matrigel matrix for organoid culture were always kept on ice and thawed as needed just before use with pre-chilled tips and tubes. In this study, organoids were cultured in three lots of Corning Matrigel matrix for organoid culture.

Mouse Intestinal Organoid Culture and Passaging

Cryopreserved mouse intestinal organoids (STEMCELL Technologies) were thawed, cultured, and passaged in IntestiCult organoid growth medium (STEMCELL Technologies) according to manufacturer's instructions. Briefly, a vial of cryopreserved MIO segments was thawed, centrifuged, and the organoid pellet was resuspended in cold IntestiCult organoid growth medium (100 µL). Then, cold Corning Matrigel matrix for organoid culture (100 µL) was added to the MIOs and the suspension was pipetted up and down. Using a prewetted 200 µL pipet tip, domes were formed in each well by adding 50 µL of the 1:1 MIO/Corning Matrigel matrix suspension to a preincubated Costar® 24-well plate (Corning). The plate, with organoid containing domes, was then placed in a humidified incubator (37°C and 5% CO₂) for 10 minutes after which IntestiCult organoid growth medium (750 µL) was added gently to each well. The culture medium was changed 3 times per week. The morphology of the MIOs was observed daily using an EVOS® XL Cell Imaging System (Thermo Fisher). Organoids were passaged after 5 to 7 days using Gentle Cell Dissociation Reagent (STEMCELL Technologies) and cultured in Corning Matrigel matrix for organoid culture domes as described above.

Organoid Immunohistochemistry

Corning Matrigel matrix domes containing MIOs were washed with Dulbecco's phosphate buffered saline (DPBS, Thermo Fisher) 3 times. Paraformaldehyde (4% in DPBS; EM Scientific) was added to the wells and incubated for 1 hour at room temperature. The wells were then washed with DPBS (3 times) to remove the paraformaldehyde. After the final rinse, DPBS containing bovine serum albumin (1%; Sigma Aldrich) was added to the wells (to prevent non-specific binding of the organoids to the wells and pipet tips), and the domes were mechanically disrupted by pipetting up and down. The organoids and organoid fragments (in 1% BSA solution) were transferred to a 96-well clear round bottom Ultra-Low Attachment microplate (Corning), covered with 96-well sealing mats, and transported

for embedding at the University of New England (Biddeford, ME).

The MIOs were embedded into agarose, then paraffin-embedded and sectioned at 5 to 8 μm . Sections were stained using hematoxylin & eosin (H&E; VWR) and immunohistochemistry (IHC) against 6 markers [lysozyme (Abcam), MUC2 (Genetex), chromogranin-A, villin, vimentin (Abcam), and E-cadherin (BD Biosciences)]; plus, isotype and primary antibody omission controls (Abcam) via multiplex immunofluorescence. H&E slides were imaged using brightfield microscopy (Keyence BZ-X700 microscope); IHC slides were assessed/imaged on an epifluorescence microscope (Leica DM2500), then final images were collected using a laser scanning confocal microscope (Leica TCS SP5).

Results and Discussion

Mouse intestinal organoids (MIOs) were cultured in domes containing a 1:1 mixture of Corning® Matrigel® matrix for organoid culture, using the HUB Organoid Technology method and IntestiCult™ organoid growth medium. The MIOs cultured in three different lots of Corning Matrigel matrix for organoid culture showed typical intestinal organoid morphology with buds (Figure 1A). Furthermore, H&E staining of embedded sections of MIOs cultured in three lots of Corning Matrigel matrix for organoid culture (Lot 1: passage 7; Lot 2: passage 9; Lot 3: passage 7) showed highly organized structures with a lumen and a budding morphology (Figure 1B).

Immunohistochemistry revealed that the MIOs cultured in all three lots of Corning Matrigel matrix for organoid culture contained differentiated intestinal cells such as mesenchymal cells, goblet cells, enterocytes, enteroendocrine cells, and paneth cells as demonstrated by the presence of vimentin, mucin2, villin, chromogranin-A, and lysozyme respectively (Figure 2).

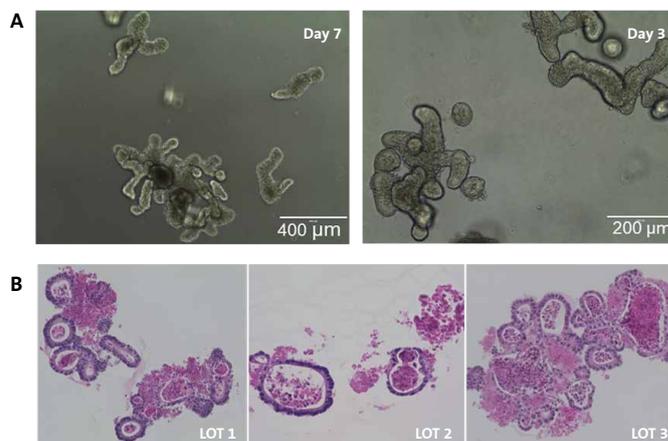


Figure 1: Morphology of mouse intestinal organoids cultured in Corning Matrigel matrix for organoid culture. (A) Representative images of MIOs in culture. (B) Representative photomicrographs of paraffin embedded and sectioned Day 7 MIOs cultured in three lots of Corning Matrigel matrix for organoid culture (Lot 1: passage 7; Lot 2: passage 9; Lot 3: passage 7).

Conclusions

- ▶ Corning Matrigel matrix for organoid culture is an optimal extracellular matrix that saves reagents and time used for lot testing. Each lot of Corning Matrigel matrix for organoid culture has a specific elastic modulus value indicating the stiffness of the gel formed and is qualified to form stable 3D domes commonly used in organoid culture protocols.
- ▶ Corning Matrigel matrix for organoid culture supported the culture and expansion of mouse intestinal organoids in IntestiCult organoid growth medium for more than 7 passages. Throughout the culture period on Corning Matrigel matrix for organoid culture, the organoids displayed typical morphology. The MIOs contained differentiated intestinal cells as demonstrated by the expression of surface markers via immunohistochemistry.

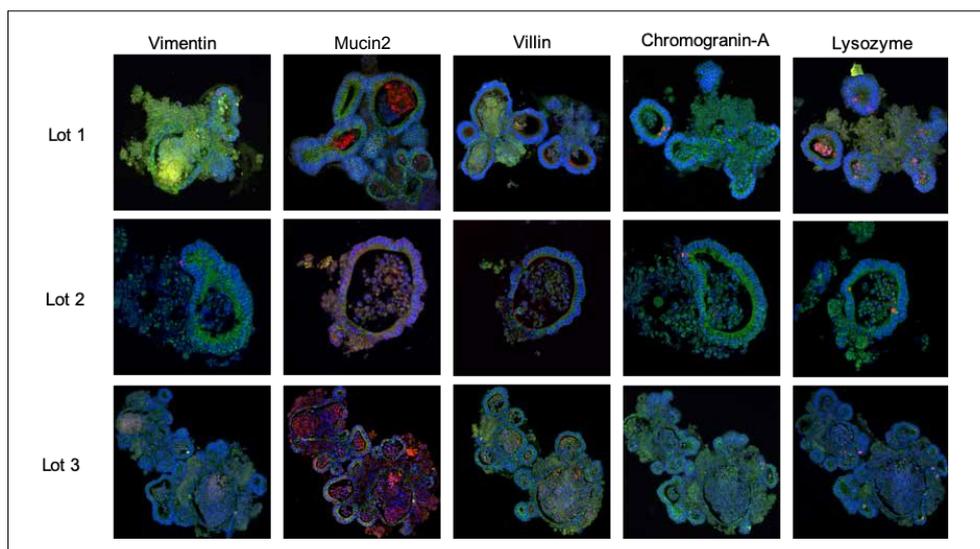


Figure 2: Representative immunofluorescent images of MIOs in three different lots (Lot 1: passage 7; Lot 2: passage 9; Lot 3: passage 7) of Corning Matrigel matrix for organoid culture show presence of vimentin, mucin2, villin, chromogranin-A, and lysozyme, demonstrating the presence of mesenchymal cells, goblet cells, enterocytes, enteroendocrine cells, and paneth cells, respectively. In each image – Green: E-cadherin; Blue: DAPI, and Red: Specific marker.

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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

1. Artegiani B and Clevers H. Use and application of 3D-organoid technology, *Human Molecular Genetics*, 2018, 27(R2):R99-R107.
2. Simian M and Bissell MJ. Organoids: A historical perspective of thinking in three dimensions, *Journal of Cell Biology*, 2017, 216(1):31-40.
3. Yin X, et al. Engineering stem cell organoids, *Cell Stem Cell*, 2016, 18:25-38.
4. Barker N, et al. Identification of stem cells in small intestine and colon by a marker gene LGR5, *Nature* 2007, 449:1003-1007.
5. Sato T, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, *Nature*, 2009, 459:262-265.
6. Kleinman HK, et al. Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma, *Biochemistry*, 1982, 21:6188-6193.
7. Kleinman HK, et al. Basement membrane complexes with biological activity, *Biochemistry*, 1986, 25:312-318.
8. Kleinman HK and Martin GR. Matrigel: Basement membrane matrix with biological activity, *Seminars in Cancer Biology*, 2005, 378-386.
9. Sanyal S. Culture and assay systems used for 3D cell culture (Corning Lit. Code CLS-AC-AN-245).

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