Corning® Matrigel® Matrix-3D Plates for High Throughput 3D Assays

Application Note

CORNING

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Introduction

Polarized epithelial cells form barriers that separate the inner and outer layers of various organs such as the kidney, colon, lungs, and mammary glands These barriers are essential for controlling passage of nutrients, hormones, gases, and cells between different parts of the body; thus, the ability of epithelial cells to polarize and form a barrier plays an important role in organ function[1]. In the body, basement membrane is responsible for helping cells to establish polarity[2]. This process can be modelled 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 a wide variety of 3D models, such as kidney cysts for studying diseases like polycystic kidney disease[3]. When kidney cysts are exposed to forskolin, they swell due to increased level of intracellular cAMP, making the cysts a useful model for polycystic kidney disease[4]. To increase the throughput for screening with 3D models, Corning has developed 96- and 384-well microplates pre-coated with Corning Matrigel matrix specifically for 3D cell culture. These plates are easy-to-use and provide the convenience of pre-dispensed Matrigel matrix in high-throughput formats while providing the consistency required for such assays. The current study highlights the use of pre-coated Matrigel matrix-3D plates to generate MDCK cysts for screening modulators of forskolin-induced cyst swelling, which can be used to identify potential therapies for polycystic kidney disease.

Materials and Methods

MDCK Cyst Culture

MDCK cells (ATCC® CCL-34™) were cultured in DMEM (Corning 10-013-CM) containing 10% FBS (Corning 35-010-CV). Twenty-four hours prior to use, Corning Matrigel matrix-3D plates (Corning 356256) were thawed by placing at 2°C to 8°C. On the day of seed, plates were transferred to 37°C to polymerize for at least 1 hour. While plates were polymerizing, cells were harvested with Accutase (Corning 25-058-CI) and resuspended at a density of 12,500 cell/mL supplemented with Corning Matrigel matrix (Corning 356231) to a concentration of 0.2 mg/mL. Once plates were polymerized, 40 µL of cell suspension was added to each well and plates were incubated for 96 hours.

MDCK Cyst Staining

Cysts were collected from Matrigel matrix by pipetting up and down with wide bore tips (Corning TF-205-WB-R-S) and incubating with Corning cell recovery solution (Corning 354253) at 4°C for 20 minutes. Cysts were washed several times with cold PBS before fixing with cold 4% paraformaldehyde for 15 minutes at 2°C to 8°C. After fixation, cysts were permeabilized with 0.5% Triton™ X for 20 minutes prior to washing with PBS and staining. Cysts were stained overnight at 2°C to 8°C in 200 µL of flow cytometry staining buffer (R&D Systems FC001) containing 1 drop of NucBlue® Fixed Cell ReadyProbes® Reagent (Thermo Fisher R37606), 2 µL of ZO1 (Thermo Fisher 339188), and 5 µL of phalloidin 647 (Thermo Fisher A22287).

Swelling Assays

Z' factor

After 96 hours, 40 µL of media containing 20 µM forskolin (MilliporeSigma F6886) or DMSO (Corning 25-950-CQC) control was added to each well. A time zero scan with the CellInsight™ CX7 confocal imager was taken and then plates were incubated for an additional 48 hours prior to scanning again.

Screen

Compounds from the Tocris™ Kinase Library (Tocris 3514) were added to MDCK plates to achieve a final concentration in each well of 10 µM forskolin and/or 10 µM compound or media alone. DMSO concentration was matched in all wells to 0.3%. Plates were imaged prior to compound addition and again after 48 hours of incubation. Several follow up hits were assessed at different concentrations following the same procedure described previously with exception to an increase in final DMSO concentration to 0.9%.

Results and Discussion

To study physiological response of an epithelial model, it is important to have the proper polarity. Figure 1 demonstrates lumen formation and appropriate apical orientation of tight junction protein ZO1 stained MDCK cysts. Functionality of the assay was demonstrated by exposing wells of MDCK cysts to 10 µM forskolin or media at the same DMSO concentration. Images in Figure 2 show distinct swelling after 48 hours with cultures that were exposed to forskolin as compared to cultures with media and DMSO. Z' factor was calculated to ensure the assay was robust (Figure 3). A screen of 80 compounds was used to screen for modulators of forskolin induced swelling. Data from 3 independent screens was averaged, normalized to media with DMSO response, and sorted by size of response (Figure 4). Four compounds that appeared to have an inhibitory effect on forskolin-induced swelling were used for follow-up tests at different concentrations. The results of follow up tests are shown in Figures 5 and 6.

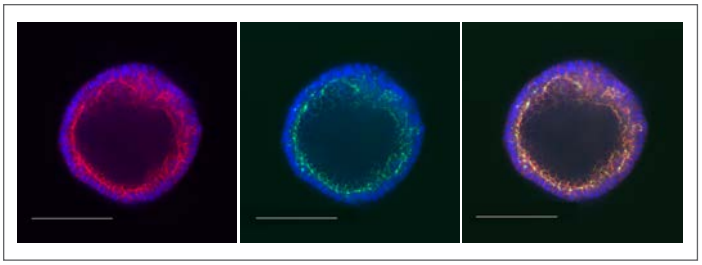

Figure 1. MDCK cyst polarity. Representative photomicrographs of fluorescently stained MDCK cysts using a 20X objective. Blue is nuclei, red is phalloidin, and green is ZO1. Right image is overlay. Scale bar is 100 µm.

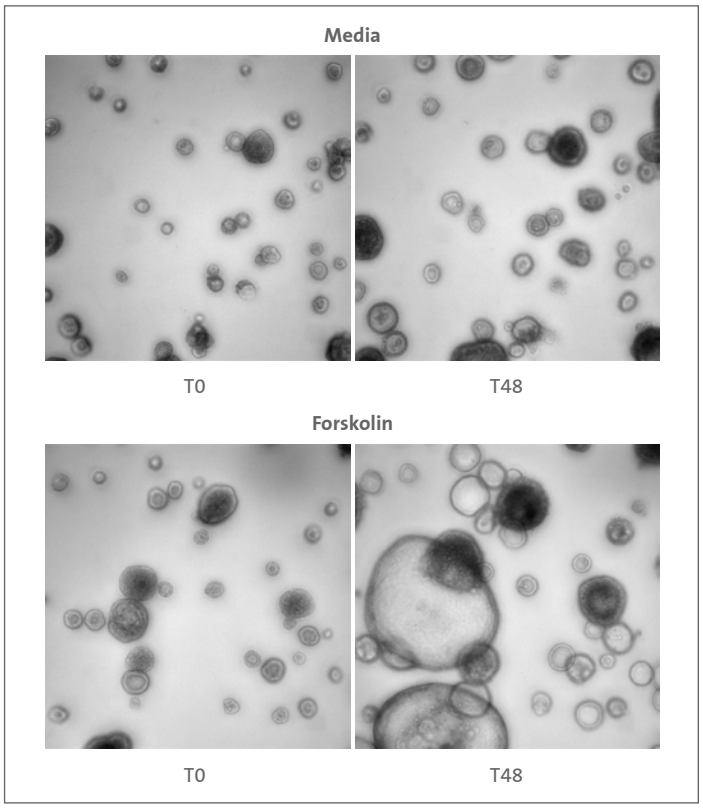

Figure 2. MDCK cysts after exposure to forskolin or media control. Representative photomicrographs of MDCK cysts after 48-hour exposure to 10 µM forskolin or media. Images taken using 10X objective from the CellInsight CX7 confocal imager.

Figure 3. Z' factor. Z' factor calculated from comparing swelling of MDCK cysts exposed to forskolin or media control. N = 16 wells per plate from 3 independent studies.

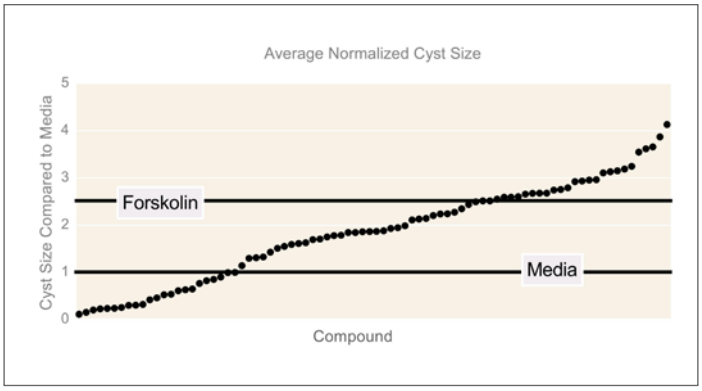

Figure 4. Tocris kinase library screen. Average change in cyst area of screened compounds as a ratio of media response. Data is average of 4 wells per compound from 3 independent screens and sorted by effect on cyst size. Media and forskolin responses are marked for reference.

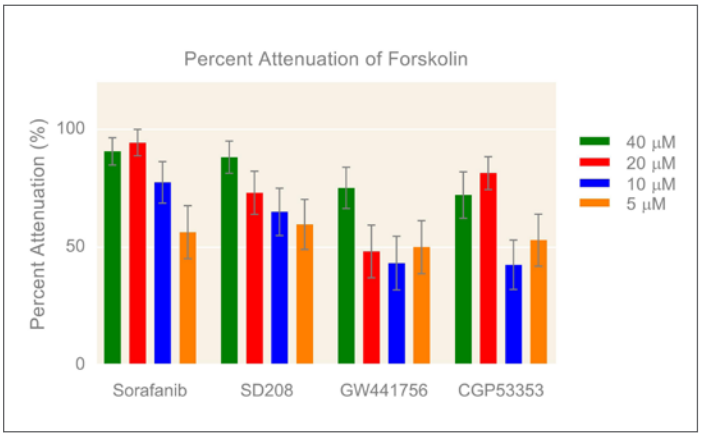

Figure 5. Follow up testing of selected hits. Follow up testing of selected hits at several concentrations resulting in percent attenuation of forskolin response. Data shown is standard error from 3 independent studies. N = 18 per dose.

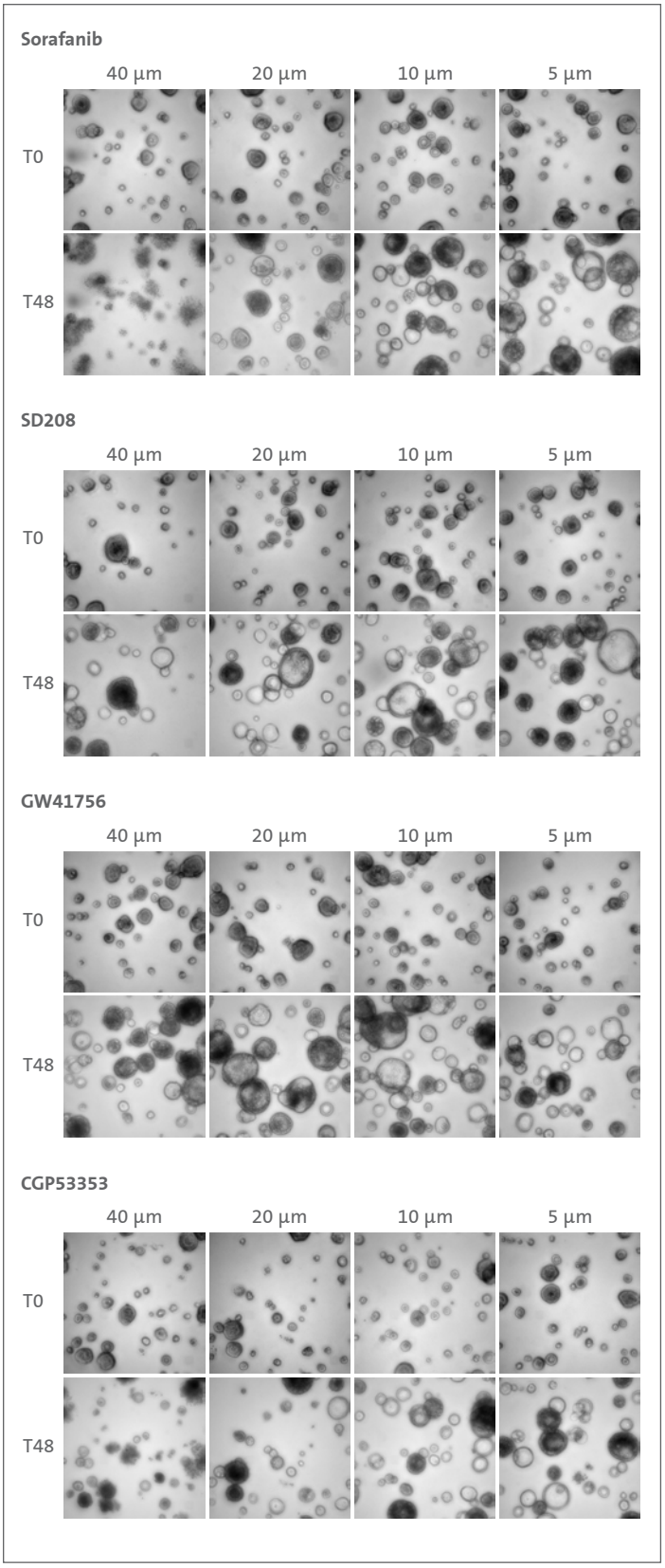

Figure 6. Representative images of follow up testing of selected hits. Representative photomicrographs of forskolin swelling response after exposure to potential inhibitors at several concentrations.

Conclusions

- Corning® Matrigel® matrix is an important tool for 3D cell culture; cells in this ECM environment successfully establish the proper polarity while forming 3D structures.
- Corning Matrigel matrix-3D plates provide a relevant biological environment for your cell assay needs without the need to self-coat.
- Corning Matrigel matrix-3D plates provide an easier work flow for higher throughput 3D applications.
- Corning Matrigel matrix-3D plates provide the robustness and consistency for high throughput assays.

References

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4. Kakade VR, et al. A cAMP and CREB-mediated feed-forward mechanism regulates GSK3 ß in polycystic kidney disease. J. Mol. Cell Biol. 2016 8(6):464-476.

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