Automation of Forskolin-induced Swelling Assay of Human Intestinal Organoids

Application Note

CORNING

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Introduction

Over the last several years, there has been increased interest around the use of patient-derived organoids for personalized medicine and drug discovery[1]. The unique ability of organoids to recapitulate the organ structure, model disease, and maintain the genetic diversity of the donor tissue makes them a promising tool[2]. In order to reach their full research potential, organoids need to be amenable to more automated and high throughput methods. Automating the handling of organoids has been problematic due to the required manipulation of viscous basement membrane extracts. Here, we demonstrate the use of the SPT Labtech dragonfly® discovery to accurately and evenly dispense small (3 µL) droplets of human intestinal organoids mixed with Corning® Matrigel® matrix for organoid culture, followed by a forskolin-induced swelling (FIS) assay.

Materials and Methods

Human intestinal organoids (HIO) from a healthy and cystic fibrosis (CF) donor carrying F508del/S1251N mutation, were purchased from Hubrecht Organoid Technology (HUB; Utrecht, Netherlands). Organoids were cultured per HUB methodologies[3]. In brief, organoids were resuspended in Corning Matrigel matrix for organoid culture (Corning 356255) and growth medium was replaced every 2 to 3 days. When organoids were ready for passage, domes were collected by pipetting with Axygen® Maxymum® Recovery 1000 µL tips (Corning T-1000-C-L-R-S). 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 14-955-456), a modification of the HUB method. For a more detailed protocol and materials list for culturing HIO please refer to "Culturing Human Intestinal Organoids with Corning Matrigel Matrix for Organoid Culture" (Corning Application Note CLS-AN-569). At least 24-hours prior to assay setup, Corning 96-well cell culture microplates (Corning 3596) were pre-warmed by placing at 37°C. Additionally, SPT Labtech reservoirs (SPT 4150-07203, 4150-07204), cool block (SPT 3152-02011), and syringes (SPT 4150-07209) were placed at -20°C. On the day of seeding, organoids were sheared to the desired size and combined with Corning Matrigel matrix for organoid culture to a ratio of 50% Matrigel matrix:cell volume. Then, the dragonfly discovery was used to dispense a single 3 µL drop into the center of each well of a 96-well microplate. There was no pipetting performed to mix while dispensing into a single plate. A full 96-well microplate took less than 1 minute to seed. Next, Matrigel matrix domes were polymerized for 15 minutes at 37°C before adding 100 µL of complete medium containing 10 µM Rock inhibitor (MilliporeSigma Y0503) with or without 3 µM VX809 (Selleckchem S1565) to each well. Sixteen to twenty-four hours later, dragonfly discovery was used to add 10 µL per well of 0.04 µg/mL Calcein AM (Corning 354216) diluted in medium. Organoids were incubated for 30 minutes until completely stained. After staining, medium was removed and replaced with 100 µL of medium containing 0.128 µM Forskolin (MilliporeSigma F6886) with or without 3 µM VX770 (Selleckchem S1144). Images of organoids were taken just prior to addition of compounds and every 10 minutes after for 60 minutes using a 2X objective Thermo Scientific CellInsight™ CX7.

Results and Discussion

In order to automate organoid assays, such as the FIS assay, it is essential that liquid handlers can accurately dispense organoids. Additionally, if the end point of the assay requires image capture, the location of dispensed volumes need to be consistent in order to keep imager scan times manageable. Figure 1 shows a typical 96-well microplate of stained human intestinal organoids dispensed in 3 µL drops using dragonfly discovery. Each well is a single and centered field of view demonstrating the ability of

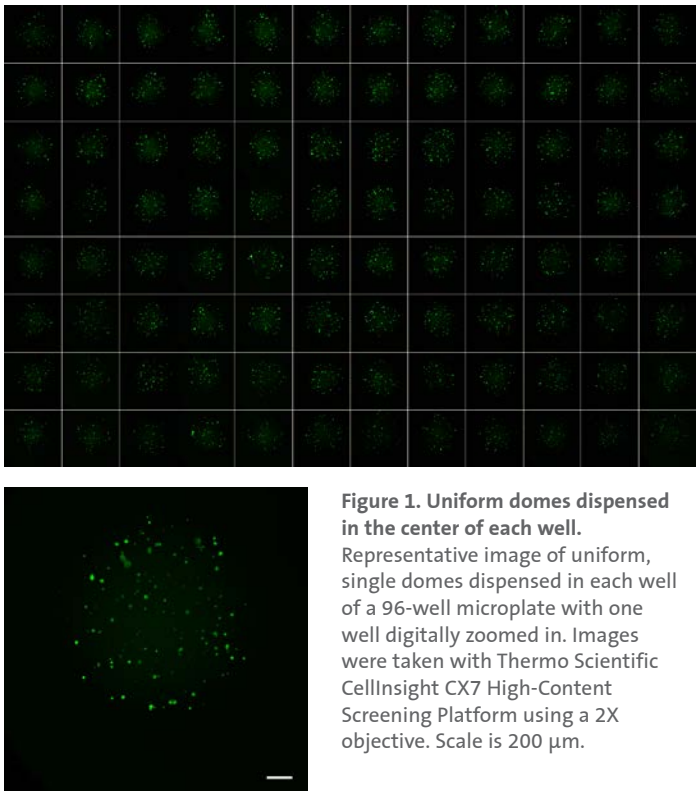

Figure 1. Uniform domes dispensed in the center of each well. Representative image of uniform, single domes dispensed in each well of a 96-well microplate with one well digitally zoomed in. Images were taken with Thermo Scientific CellInsight CX7 High-Content Screening Platform using a 2X objective. Scale is 200 µm.

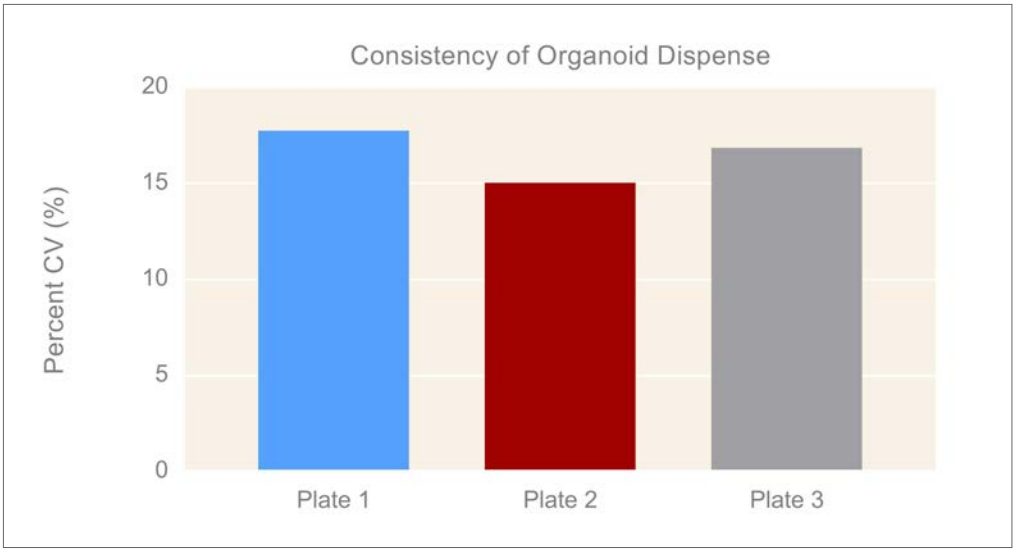

Figure 2. Consistency of dispense within a plate. The number of organoids dispensed in each dome from three 96-well microplates was enumerated in order to calculate consistency across each plate. Each plate resulted in a CV of less than 18%.

the instrument to precisely dispense drops of the same size in a consistent location. Further, the instrument dispenses a consistent number of organoids in each drop as shown by coefficients of variation (CV) (in Figure 2). The FIS assay was chosen as proof of the capability to automate a functional organoid assay. The FIS assay is used to detect the presence of mutations in the encoding cystic fibrosis transmembrane conductance regulator (CFTR) and potential therapies to correct the disfunction. The premise is that mutations in CFTR cause improper regulation of fluid and electrolytes in epithelial cells of organs, such as the intestine and the lung[4]. Healthy HIO swell in size when stimulated with forskolin due to an increase of fluid secretion in the lumen of the organoid. CF organoids, depending on the extent of the mutation, demonstrate more limited or no swelling when stimulated with forskolin. Part of the challenge with finding treatments for CF is that there are many different CFTR mutations and the mutation will dictate the best drug combination for each patient[3].

Figure 3 shows representative images of organoids prior to stimulation and 60 minutes after with or without the addition of treatment(s). The images show the difference in response of healthy organoids compared to CF organoids with DMSO and no additional drugs. The healthy organoids increase in size with a noticeably larger lumen in many of the organoids after 60 minutes. There seems to be little to no noticeable change in CF organoid size when DMSO or VX809 (a CFTR corrector that partially restores CFTR function with some CFTR mutations) is added[5]. VX770 (a CFTR potentiator that has been shown to increase the activity CFTR proteins) appears to have a positive impact on CF organoid swelling. Figure 4 is the average measured change in organoid size from 2 independent studies. The data shows that the combination of VX770 and VX809 is a potential treatment for this patient's specific CFTR mutation. Additionally, the data is consistent with what has been previously reported for CF lines with a F508del/S1251N mutation.

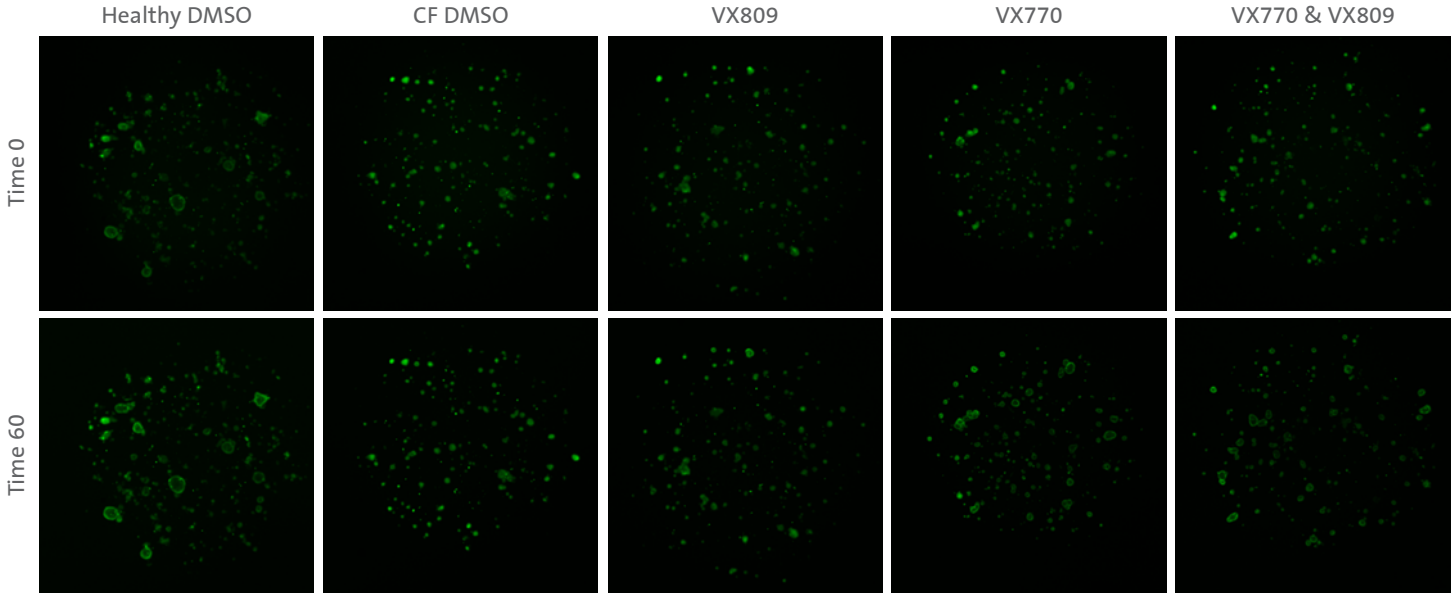

Figure 3. Representative images before and after forskolin addition. Representative photomicrographs demonstrating healthy and CF organoid swelling response under different conditions. Images were taken with Thermo Scientific CellInsight CX7 High-Content Screening Platform using a 2X objective.

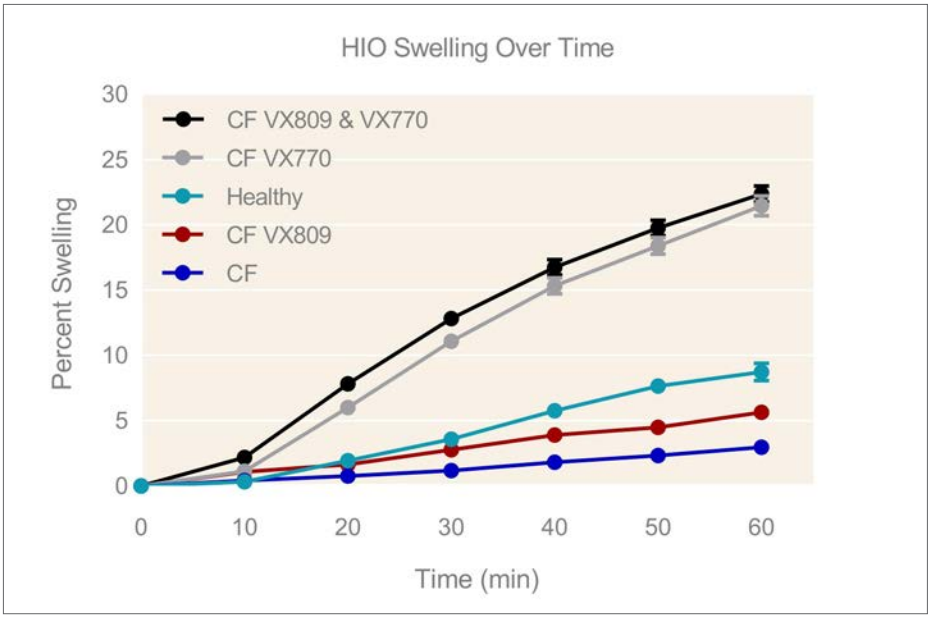

Figure 4. Forskolin-induced HIO swelling over time. Average percent swelling compared to time 0 of healthy and CF HIO. Data is the average from two independent studies shown with standard error bars. N = 38 for healthy HIO and 40 for CF HIO conditions.

Conclusions

Automating the handling of organoids is essential for organoids to become a feasible tool for personalized medicine and drug discovery. The dragonfly discovery is a positive displacement, non-contact dispenser that can accurately and precisely dispense low volumes. By pre-chilling all consumables, we were able to dispense 3 µL droplets of human intestinal organoids suspended in Corning® Matrigel® matrix for organoid culture into 96-well microplates. The dispensed organoids are functional and can be used in a wide variety of assays such as the FIS assay demonstrated in this application note.

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

References

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