Liquid Handling in a 96-well Corning® Elplasia® Plate using the INTEGRA VIAFLO 96/384

Application Note

CORNING

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Introduction

The use of three-dimensional (3D) cell culture models in cancer research and drug discovery has grown in recent years. 3D cell culture models offer more physiologically relevant models than traditional 2D cell culture models. For example, 3D cell cultures can maintain the original shape, polarity, genotype, and heterogeneity observed *in vivo*.[1] The ability to consistently form and assay spheroids has been improved by the availability of products such as the Corning spheroid microplate, which enables the formation of a single spheroid per well in an assay-compatible microplate. However, some assays require a larger number of data points or more assay signal than one spheroid can generate. Corning Elplasia plates contain microcavities within each well and an Ultra-Low Attachment (ULA) surface coating to enable the formation of multiple spheroids per well ranging from 79 spheroids in the 96-well Elplasia plate to up to 2,885 spheroids in the 6-well Elplasia plate. Some applications require that a media change or wash step be performed within the Elplasia plate, which can be difficult to do without causing spheroid loss from the microcavities. This process is made easier through the use of automation. The INTEGRA VIAFLO 96/384 is a compact handheld electronic pipettor that is easy to use. It features a 24-, 96-, or 384-channel head and is simple to use, allowing for fast and precise multi-channel pipetting. The VIAFLO 96/384 can also be programmed easily so that each pipet tip can go to the same location and height within a well each time, which is particularly important when working with the Elplasia plates.

We have created protocols using the INTEGRA VIAFLO 96/384 for seeding cells and for performing media change or wash steps in 96-well Elplasia plates. Following the methods described in this paper cell seeding with the VIAFLO 96/384 resulted in low variability in spheroid size as represented by low %CV values. By using the VIAFLO 96/384 to perform multiple wash steps, spheroid loss was minimized and >95% of spheroids within a 96-well Elplasia plate were retained. Additional optimization and validation may be required for alternate cell types and densities.

Materials and Methods

The cells lines HT-29/GFP (Creative Biogene CSC-RR0119), HepG2 (ATCC® HB-8065™), and A549 (ATCC® CCL-185™) were thawed and cultured following vendors' protocols. Cells were harvested using 0.05% Trypsin, 0.53 mM EDTA (Corning 25-052-CV) and were resuspended in their recommended culture media. The seeding and media change tests were each performed with one microplate three independent times.

INTEGRA VIAFLO 96 loaded with 300 µL GripTips with a 96-well Corning Elplasia plate

Cell Seeding

Each Corning Elplasia 96-well plate (Corning 4442) was pre-wet with 50 µL per well of McCoy's 5A medium (Corning 10-050-CV) supplemented with 10% fetal bovine serum (FBS; Corning 35-010-CV) using an INTEGRA VIAFLO 96/384 and a 10 to 300 µL pipetting head. The plate was centrifuged at 500 x g for 1 minute to remove air bubbles prior to cell seeding. HT-29/GFP cells were seeded in 100 µL/well at 500 cells per microcavity using the VIAFLO 96/384. The cells were incubated in a 37°C, 5% CO_2 humidified incubator for 1 day to allow for spheroid formation. The next day, the Elplasia plate was imaged using a Thermo Fisher CellInsight CX7 HCS platform with a 4X objective. For each well, images were taken with brightfield mode to image the microcavities and with a green fluorescent channel to image the cells. The images of each well were analyzed by measuring the average diameter of the spheroids. The same plate was then used for a CellTiter-Glo® 3D cell viability assay (Promega G9682) to determine the relative number of cells. To each well containing 150 µL of culture media,

150 µL of reagent was added. The Elplasia plate was shaken for 5 minutes and incubated in the dark at room temperature for 25 minutes. Luminescence was detected with a PerkinElmer EnVision multimode plate reader.

Media Change

Each Corning Elplasia 96-well plate was pre-wet with 50 µL of culture media and centrifuged at 500 x g for 1 minute to remove air bubbles prior to cell seeding. The HT29/GFP, HepG2, and A549 cell lines were each seeded in 100 µL/well at the following seeding densities into one column per density: 125, 250, 500, and 1,000 cells per microcavity. The culture medium for HepG2 cells composed of MEM (Corning 10-010-CV) supplemented with 10% FBS and the culture medium for A549 cells composed of F-12K (Corning 10-025-CV) supplemented with 10% FBS. The cells were incubated in a 37°C, 5% CO_2 humidified incubator for 1 day to allow for spheroid formation.

The next day, cell nuclei were stained by using an INTEGRA VIAFLO 96/384 and a 10 to 300 µL pipetting head to add 20 µL of Hoechst 34580 (Thermo Fisher H21486) for a final concentration of 10 µg/mL. The cells were incubated in a 37°C, 5% CO_2 humidified incubator for 30 minutes to allow time for the stain to penetrate the spheroids. The Elplasia plate was then imaged using a CellInsight CX7 HCS platform with a 4X objective to establish a baseline. For each well, images were taken with brightfield mode to image the microcavities and with a blue fluorescent channel to image the cell nuclei. The images of each well were analyzed by counting the average number of objects (spheroids) within each microcavity.

Each Elplasia plate was washed using an INTEGRA VIAFLO 96/384 and a 10 to 300 µL pipetting head. First, culture media was removed leaving 50 µL per well, which is the minimum volume required to fill the microcavities. Then 150 µL per well of DPBS without calcium and magnesium (Corning 21-031-CM) was added. Each plate was washed four times and imaged with the CellInsight CX7 HCS platform after each wash to monitor spheroid loss.

Results and Discussion

Cell Seeding

GFP-expressing HT-29 cells were seeded into a 96-well Elplasia plate at 500 cells per microcavity using an INTEGRA VIAFLO 96/384. The ability of the VIAFLO 96/384 to precisely dispense the same volume of cells into each well was assessed using high content imaging and a cell viability assay.

For high content imaging, a CellInsight CX7 HCS platform was used to take images of the spheroids in each cavity using brightfield and green fluorescent channels (Figure 1).

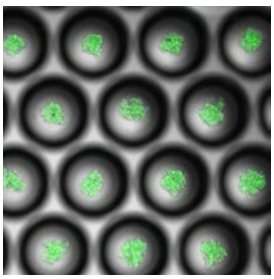

Figure 1. Seeding HT-29/GFP cells using an INTEGRA VIAFLO 96/384 resulted in consistent, single spheroids in each microcavity. Representative image of HT-29/GFP cells seeded at 500 cells per microcavity in a 96-well Corning Elplasia plate. Image was taken with a CellInsight CX7 confocal imager using a 4X objective with one field digitally zoomed in.

The average spheroid diameter (object size) for each well was determined and used to calculate a precision value (%CV) for each Elplasia plate. For each of the three independent studies, the %CV values were below 10%, demonstrating high precision (Figure 2A).

After high content imaging, the same microplates were used for a CellTiter-Glo 3D cell viability assay. The resulting luminescence from each well was used to calculate %CV values for each Elplasia plate. For each of the three independent studies, the %CV values were below 10%, demonstrating high precision (Figure 2B).

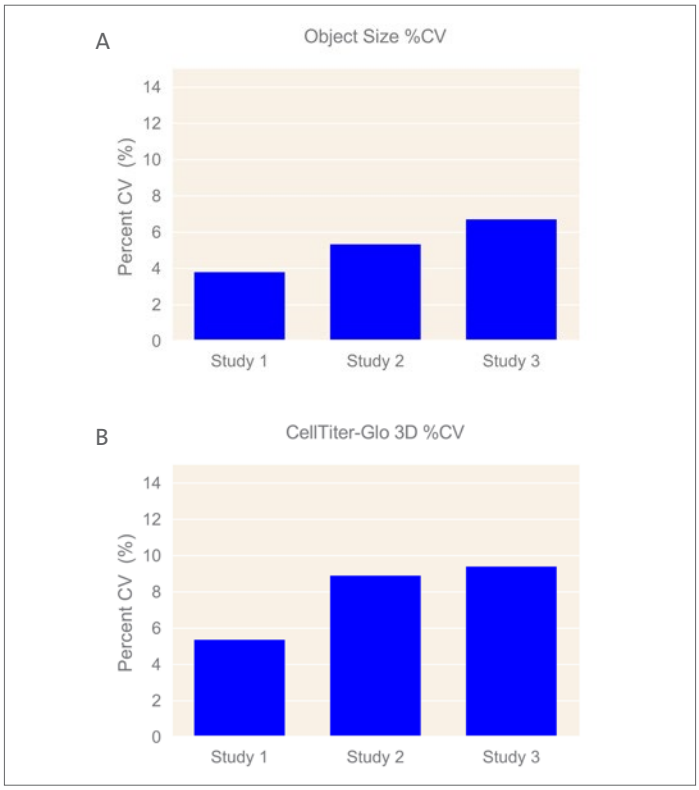

Figure 2. Low percent CV values were achieved for cell seeding using an INTEGRA VIAFLO 96/384. HT-29/GFP cells were seeded into a 96-well Corning Elplasia plate with an INTEGRA VIAFLO 96/384 on three different days. (A) After overnight incubation, the average spheroid diameter (object size) in each well was determined using a CellInsight CX7 HCS platform and was used to calculate a %CV value for each plate. (B) The same plate was then used for a CellTiter-Glo 3D luminescent assay and the resulting luminescence was used to calculate a %CV value for each plate.

Media Change

To assess the ability of the VIAFLO 96/384 to perform media change steps in 96-well Elplasia plates, three different cell lines that form spheroids of varying sizes were included: HT-29/GFP, HepG2, and A549 cells. The cells were seeded at 125, 250, 500, and 1,000 cells per microcavity and incubated overnight for spheroid formation prior to testing. Cell nuclei were stained with Hoechst and high content imaging with a CellInsight CX7 HCS platform was used to determine the number of microcavities that retained a single spheroid. Representative images are displayed in Figure 3. The plates were rescanned with the high content imager after each of four wash steps with a VIAFLO 96/384. The average percent of spheroids lost from each well after all four washes is displayed by cell type and density in Figure 4. For the three cell lines tested at 125 to 1,000 cells per microcavity, four washes with the VIAFLO 96/384 resulted in an average of less than 5% spheroid loss.

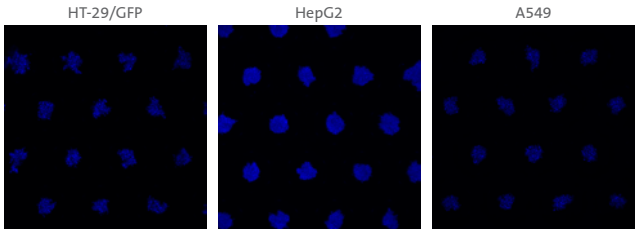

Figure 3. Three cell lines formed consistent, single spheroids in each microcavity of 96-well Corning Elplasia plates. Representative images of nuclei-stained HT-29/GFP, HepG2, and A549 cells seeded at 500 cells per microcavity in a 96-well Elplasia plate. Images were taken with a CellInsight CX7 confocal imager using a 4X objective with one field digitally zoomed in.

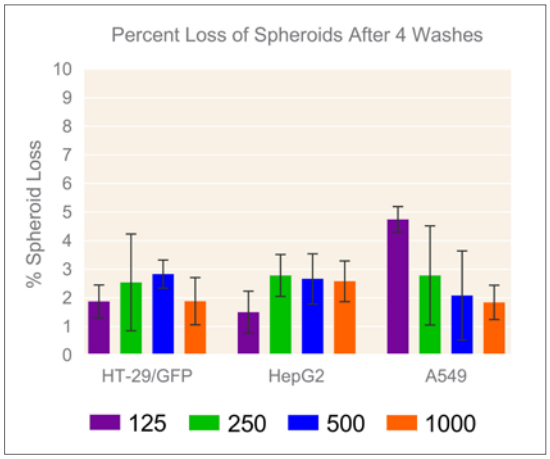

Figure 4. Four washes with an INTEGRA VIAFLO 96/384 resulted in less than 5% spheroid loss from 96-well Corning Elplasia plates. The HT-29/GFP, HepG2, and A549 cell lines were seeded at 125 to 1,000 cells per microcavity and incubated overnight. An INTEGRA VIAFLO 96/384 was used to add Hoechst stain to each well and to perform 4 wash steps by removing all but 50 µL/well and adding 150 µL/well of DPBS. Each plate was imaged using a CellInsight CX7 to count the number of spheroids per well before and after each wash. Data shown with standard error of the mean from 3 independent studies. N=24 wells.

Conclusions

- Corning Elplasia plates enable the formation of multiple, consistently-sized spheroids per well.
- The INTEGRA VIAFLO 96/384 can be used to seed precise volumes of cells into Corning Elplasia plates to meet higher throughput demands.
- Media changes and wash steps can be performed in 96-well Corning Elplasia plates using the INTEGRA VIAFLO 96/384.

References

1. Antoni D, Burckel H, Josset E, and Noel G. (2015). Three-dimensional cell culture: a breakthrough *in vivo*. International journal of molecular sciences, 16(3):5517-5527. doi:10.3390/ijms16035517.

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