Corning® Elplasia® Plates
Assays Requiring Multiple Spheroids per Well

Application Note

CORNING

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Introduction

It has been well-established that 3D cancer models are more reflective of *in vivo* tumor microenvironment when compared to traditional 2D models[1]. As a result, there is a growing trend for more representative 3D cancer cell models. Traditional scaffold-free 3D models often force researchers to choose between a) multiple spheroids per well of a non-uniform shape and size, or b) a single uniform spheroid per well. However, multiple uniform spheroids in a single well are often desired to increase assay signal or increase the number of data points under a single condition. Here we demonstrate how Corning Elplasia 96-well microplates can reproducibly form multiple uniform ovarian cancer spheroids that can be used for luminescent, fluorescent, and high content imaging assays.

Materials and Methods

Spheroid Formation

Corning Elplasia plates (Corning 4442) were pre-wet prior to seeding cells by adding 50 µL of DMEM (Corning 10-013-CM) with 10% fetal bovine serum (Corning 35-010-CV) per well and centrifuging at 500 x g for 1 min. Once all trapped air was removed, HEY-T30 (ATCC® CRL-3252™) or SKOV3 (ATCC HTB-77) cells were seeded into plates at approximately 100 cells per microcavity in a volume of 150 µL per well. Cells were incubated for 24 hours.

Spheroid Consistency

After an overnight incubation of HEY-T30 spheroid formation, 20 µL of NucRed® Live 647 ReadyProbes® Reagent (Thermo Fisher R37106) was added to each well and incubated for 20 to 30 minutes. Once cells were completely stained, spheroids were imaged with the Thermo Scientific CellInsight™ CX7 confocal imager to assess spheroid circularity and area.

Homogenous Cell-based Assay

After an overnight incubation for HEY-T30 spheroid formation, 10 µL of Triton™ X solution to achieve a final concentration of 0.5% or buffer was added into each well. Sixteen to 24 hours later, equal volume of CellTiter-GLO® 3D Cell Viability Assay (Promega G9683) was added to each well and allowed to incubate at room temperature for 30 minutes with a 5-minute shake step. Plates were then read for luminescent signal using a PerkinElmer Envision.

High Content Imaging Assay

After an overnight incubation, HEY-T30 and SKOV3 spheroids were exposed to cisplatin (Thermo Fisher 1134357) by adding 20 µL at various dilutions. The plates were incubated overnight. In order to assess cytotoxicity, 10 µL per well of 340 µg/mL Hoechst 34580 (Thermo Fisher H21486) and 136 µg/mL propidium iodide (AnaSpec 83215) diluted in phosphate buffered saline (PBS; Corning 21-030-CM) were added to each well for 1 hour. Plates were then imaged on the Thermo Scientific CellInsight™ CX7 confocal imager.

Results and Discussion

Assay Robustness

The unique design of Corning Elplasia plates promote the generation of multiple, uniform spheroids in each well (Figure 1). To assess the ability of Corning Elplasia plates to generate uniform spheroids, HEY-T30 cells were seeded into plates overnight. Once spheroids were generated, they were stained with a fluorescent dye so that circularity and consistency of the spheroids could be examined (Figure 2). The data shows that the spheroids generated were consistent in shape and size throughout the plate. In addition to consistency of spheroid generation, it is also important to be able to execute homogenous cell-based assays in Corning Elplasia plates. Figures 3 and 4 demonstrate high signal-to-background ratios (S/B) and repeatable Z' from a homogenous luminescent assay run directly in the plate.

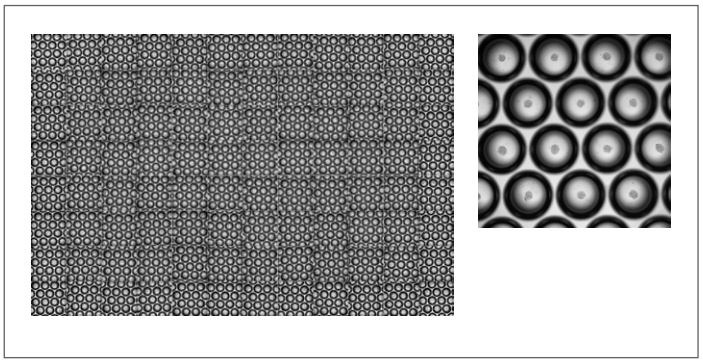

Figure 1. Uniform, single spheroids formed in each microcavity. Representative image of one field from each well of a 96-well Corning Elplasia plate with one field digitally zoomed in. Images were taken with the Thermo Scientific CellInsight CX7 confocal imager using a 4X objective.

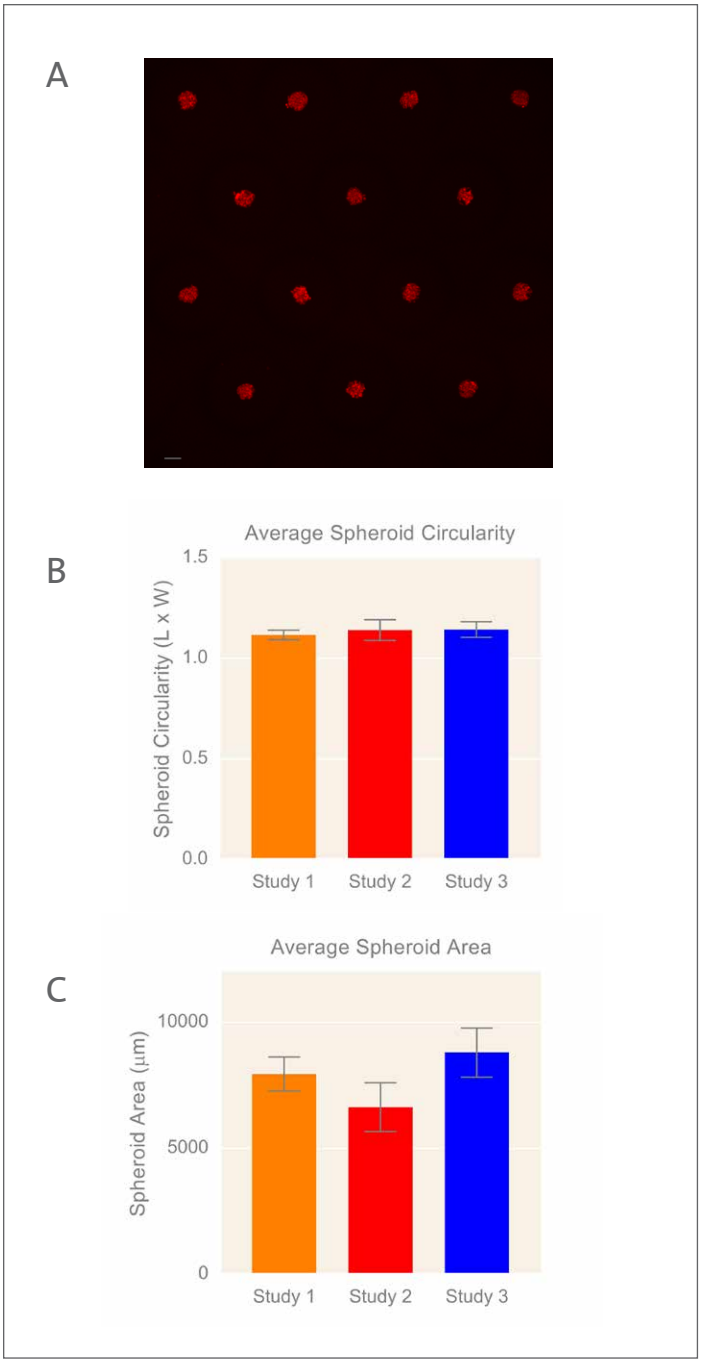

Figure 2. Uniform spheroids generated in each microcavity. Representative image of NucRed stained HEY-T30 spheroids (A) used to assess spheroid circularity (B) and average spheroid area per well (C) from several studies. Images were taken with the Thermo Scientific CellInsight CX7 confocal imager using a 4X objective. Data shown with standard deviation from 3 independent studies. N=288 wells. Scale bar is 100 µm.

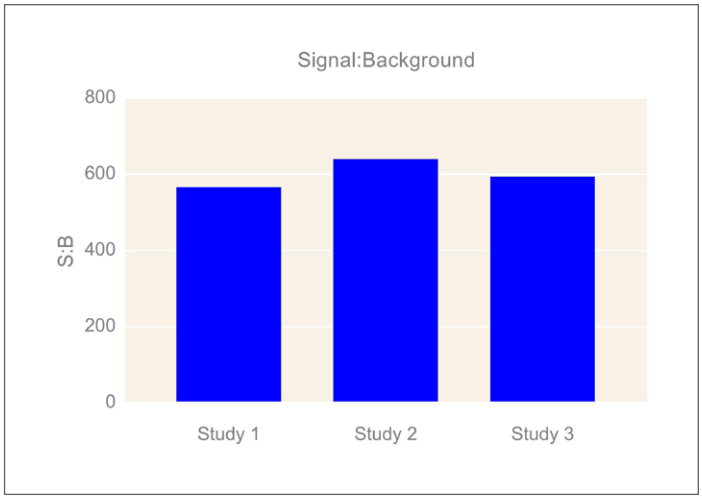

Figure 3. Large luminescence assay window. Signal-to-background ratios from 3 independent tests using HEY-T30 spheroids exposed to 0.5% Triton X or buffer.

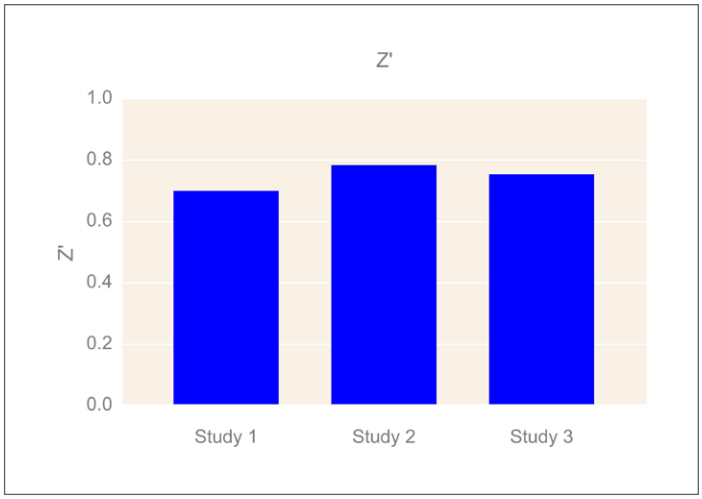

Figure 4. Excellent Z'. Z' values above 0.7 were achieved with 3 independent studies.

Proof of Concept Assay

To demonstrate Corning® Elplasia® plates utility for high content imaging applications, HEY-T30 and SKOV3 spheroids were exposed to varying concentrations of cisplatin for 24 hours. Spheroids were then stained with Hoechst and propidium iodide (PI) and imaged with the Thermo Scientific CellInsight™ CX7 high-content screening platform. Representative images were captured directly in Corning Elplasia plates, demonstrating ability of culture and subsequent imaging of spheroids (Figure 5). Image analysis of PI staining reveals concentration-response curves for cisplatin cytotoxicity with IC_{50} values of 20 µM and 9.4 µM for HEY-T30 and SKOV3 spheroids respectively, which are consistent with those reported in literature (Figure 6)[2-3].

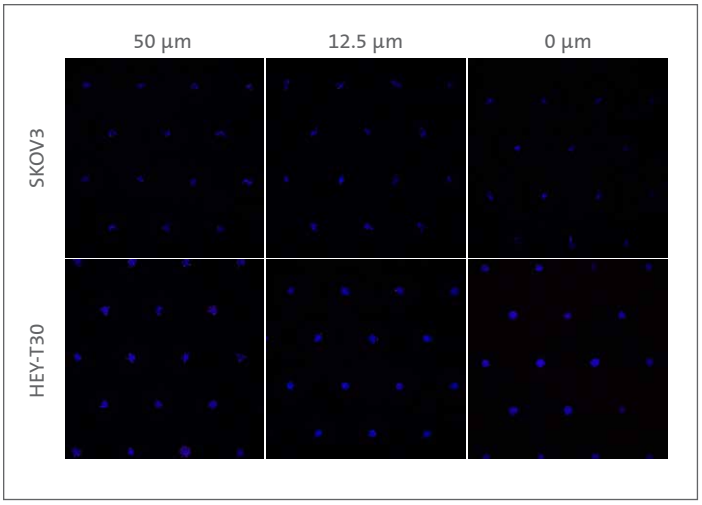

Figure 5. High content imaging of spheroids within a Corning Elplasia plate. Representative Z-stack images of SKOV3 (top) and HEY-T30 (bottom) spheroids exposed to cisplatin (50 µM left, 12.5 µM middle, and 0 µM right) for 24 hours and stained with Hoechst and propidium iodide to assess cell viability. Images taken with the Thermo Scientific CellInsight CX7 confocal imager using a 4X objective.

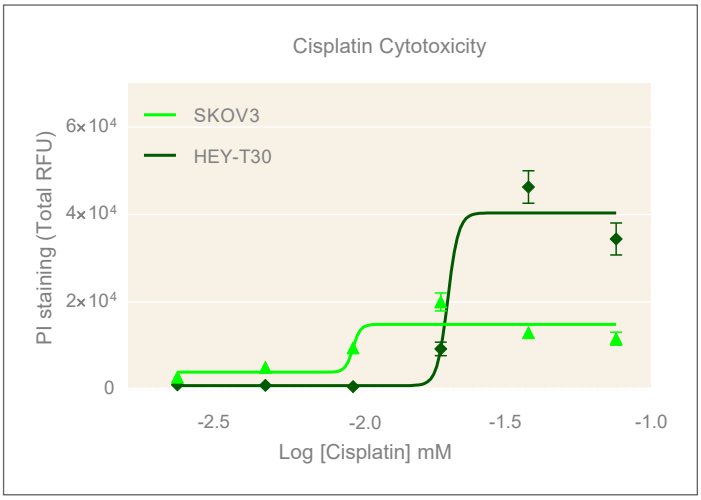

Figure 6. Concentration-dependent cisplatin cytotoxicity. Concentration-dependent responses of SKOV3 and HEY-T30 spheroids to 24 hours of cisplatin exposure at varying concentrations. Data shown is standard error from 3 independent studies. N=24 per dose. TC_{50} = 9.4 µM (SKOV3) and 20 µM (HEY-T30).

Conclusions

3D cell culture has become an important tool of the drug discovery process, which allows for research that is often more relevant than more traditional 2D cell culture. With the increase in the importance and implementation of 3D cell culture, the need for better 3D tools to generate more spheroids will also increase. Corning® Elplasia® plates provide a format to generate multiple, consistent spheroids per well for spheroid scale up or assays where more samples or larger signal is required. The black sidewalls and clear bottom design of Corning Elplasia plates make them ideally suited for imaging, luminescent, and fluorescent cell-based assays. Additionally, they are easy to use and can produce data with high signal-to-background ratios and consistent Z' values.

References

1. Imamura Yoshinori, et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncology reports 33(4); 1837-1843 (2015).
2. Song Jin, et al. Annexin XI is associated with cisplatin resistance and related to tumor recurrence in ovarian cancer patients. Clinical Cancer Research 13(22):6842-6849 (2007).
3. He Yifeng, et al. The changing 50% inhibitory concentration (IC50) of cisplatin: A pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer.

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