High-throughput, Three-Dimensional Assay Development with Corning® 1536-well Spheroid Microplates and INTEGRA Biosciences' VIAFLO 384 and VIAFILL Liquid Handlers

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Application Note

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Introduction

It is becoming more apparent that 3D models often better recapitulate the *in vivo* cell environment than traditional 2D methodologies. Accordingly, more spheroid and other 3D models are being utilized for drug discovery. To meet the demands of most high-volume drug discovery programs, a high throughput system is essential. The Corning 1536-well spheroid microplate can generate 1536 uniform, single spheroids that can be assayed via imaging, fluorescence, or luminescence directly in the microplate. With a maximum volume of 14 µL per well, accurate liquid handling is essential to the generation of quality data. We have established an automated method for seeding, dosing, and assaying spheroids in a Corning 1536-well spheroid microplate format with the assistance of INTEGRA Biosciences' liquid handling instruments. The VIAFILL is designed for rapid bulk liquid dispensing at volumes as low as 0.5 µL and can be fully automated with a plate stacker accessory. The VIAFLO 384 is a handheld electronic pipetting system which enables transfer into 24-, 96-, 384-, and 1536-well microplates in a fast, compact, and easy-to-use manner. Here we have demonstrated robust quality data for assay development and high throughput screens generated via this method.

Materials and Methods

Spheroid Formation

DU 145 (ATCC® HTB-81) and PANC-1 (ATCC CRL-1469™) cells were routinely cultured in Dulbecco's Modification of Eagle's Medium (DMEM; Corning Cat. No. 10-013-CM) containing 10% fetal bovine serum (FBS; Corning Cat. No. 35-010-CV). Cells were seeded into 1536-well spheroid microplates (Corning Cat. No. 4527) at 1 x 10^3 cells per well in a volume of 5 µL per well using the VIAFILL with the 16-channel dispensing cassette (INTEGRA Biosciences Cat. No. 5742). Microplates were incubated overnight at 37°C in a humidified CO_2 incubator to allow for spheroid formation.

Homogenous Cell-based Assay

After DU 145 spheroid formation, 1 µL of 6 µM staurosporine (MilliporeSigma Cat. No. 569396) or buffer control was dispensed into each well via the VIAFLO 384 with a 384-channel pipetting head (0.5-12.5 µL) and 12.5 µL short GripTips (INTEGRA Biosciences Cat. No. 6475). The next day, 6 µL of CellTiter-GLO® 3D (Promega Cat. No. G9683) was added to each well and allowed to incubate at room temperature for 1 hour. Luminescent signal was then read via a TECAN Infinite® M200 microplate reader.

High-content Imaging Assay

After spheroid formation, both DU 145 and PANC-1 spheroids were exposed to various concentrations of cisplatin (Sigma-Aldrich/MilliporeSigma Cat. No. 1134357) by adding 1 µL via the VIAFLO 384 with 12.5 µL short GripTips. The plates were incubated overnight. To assess cytotoxicity, 6 µL per well of 40 µg/mL Hoechst 34580 (Thermo Fisher Cat. No. H21486) and 16 µg/mL propidium iodide (PI; AnaSpec Cat. No. 83215) in phosphate buffered saline (PBS; Corning Cat. No. 21-030-CM) were added to each well for 1 hour. Microplates were than imaged on the Thermo Fisher CellInsight™ CX7 high-content screening platform.

Results

Assay Robustness

The unique design of the Corning 1536-well spheroid microplate allows for the generation of a single, uniform spheroid in each well (Figure 1). To ensure that the spheroids are of uniform size

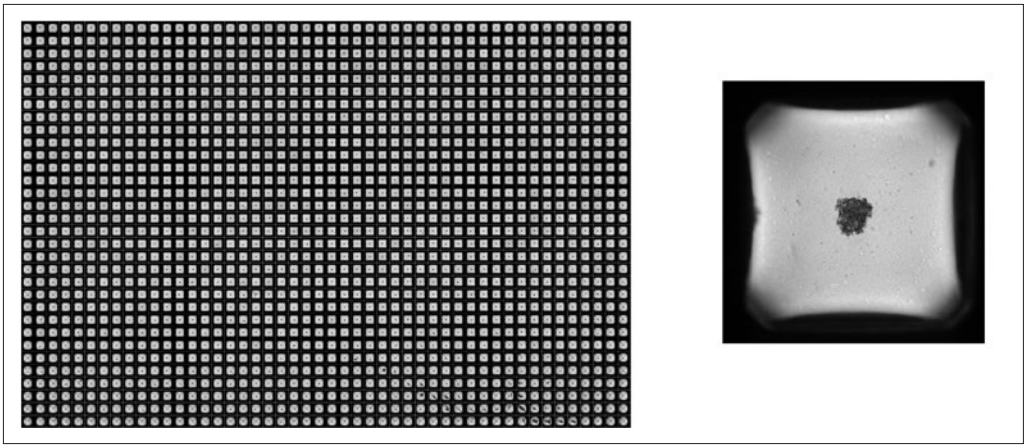

Figure 1. Uniform, single spheroids formed in each well across the entire spheroid microplate. Representative image of single uniform DU 145 spheroids formed in the Corning 1536-well spheroid microplate with one well digitally zoomed in. Images were taken with Thermo Fisher CellInsight CX7 high-content screening platform using a 4X objective.

Figure 2. Microplate dispenser for uniform cell seeding. INTEGRA VIAFILL for rapid, bulk liquid dispensing into 96-, 384-, or 1536-well microplates.

Figure 3. Electronic pipetting system for accurate compound delivery. INTEGRA VIAFLO 384 for increased productivity and efficiency to accurately work with 24-, 96-, 384-, and 1536-well microplates.

across the entire microplate, accurate liquid handling of the cell suspension during seeding is critical. For rapid cell seeding, the INTEGRA VIAFILL was utilized (Figure 2). This bulk liquid dispenser can accurately seed a 1536-well microplate in less than 1 minute per plate and can be paired with a plate stacker for increased throughput. For compound and reagent addition, the INTEGRA VIAFLO 384 was utilized. With its small footprint, the handheld electronic 24-, 96-, and 384-channel pipetting system can increase throughput and productivity in filling microplates while using disposable tips (Figure 3). The uniformity of signal, signal-to-background ratio (S/B), and calculated Z' values were assessed to demonstrate the utility of this experimental design for 3D cell screening. Uniformity of signal was demonstrated by treating sections of the microplate with a single concentration of buffer or staurosporine to induce cell death and assaying spheroid viability via homogenous luminescence cell viability assay. A heat map of luminescence signal shows good signal uniformity within treatment conditions regardless of location in the microplate (Figure 4). The mean S/B ratio from this assay was greater than 20 for 3 independent studies (Figure 5). Additionally, Z' values were consistently above 0.7, indicating the robustness of the viability assay in the 1536-well spheroid microplate (Figure 5).

Proof of Concept Assay

DU 145 and PANC-1 spheroids exposed to varying concentrations of cisplatin were stained and imaged with the Thermo Fisher CellInsight™ CX7 high-content screening platform. Representative images were captured directly without the need to transfer the spheroids to another microplate, demonstrating utility of culture and subsequent imaging of spheroids in the spheroid microplate (Figure 6). Image analysis of PI staining reveals typical concentration-response curves for cisplatin cytotoxicity with IC_{50} values of 0.03375 and 0.03917 mM for DU 145 and PANC-1 spheroids, respectively (Figure 7).

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A	206	241	316	231	351	306	206	131	131	321	301	276	176	126	211	658	6344	6565	6174	6269	6189	6119	5884	5894	6284	5799	6184	6034	5238	5964	6089	5348	246	216	186	161	106	116	201	246	251	286	206	186	171	266	226	216
B	351	276	296	291	291	346	296	246	226	236	326	216	196	291	306	243	6019	6590	5388	5784	5388	5959	5889	6134	6259	6289	6424	5909	5859	6104	6284	5238	286	366	351	426	306	246	286	281	316	236	226	251	211	211	241	151
C	306	321	291	286	296	366	331	251	296	401	311	311	226	181	241	378	5919	5604	6049	5864	6014	6865	6294	6259	5954	6580	6670	6439	6660	6444	5298	6354	361	311	271	241	196	251	221	366	251	316	211	266	271	231	186	166
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L	226	266	191	461	276	281	341	406	301	261	301	291	256	331	346	318	6149	6049	6249	5949	6174	5418	5989	6930	5839	5754	6344	6359	6134	5924	6439	5489	286	326	276	211	206	301	276	286	336	276	256	236	201	276	226	281
M	266	266	266	176	251	296	321	206	211	346	276	261	271	141	271	373	5969	6309	5944	6454	6164	6084	6524	6705	6204	5879	6089	5839	5499	5428	5203	5674	301	261	241	296	316	281	186	191	206	201	256	296	216	226	256	211
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U	7135	6565	6319	7846	7030	6840	7621	6209	6519	6645	6389	6550	5559	5989	6494	5569	306	251	281	296	166	301	261	306	181	221	276	296	351	281	301	348	6630	6284	5799	6129	5639	6219	5083	6850	6404	6214	6279	5869	6630	6605	5519	6244
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Y	7075	7380	7055	6364	6800	7415	7100	6690	5884	6519	6690	6094	5599	6329	6104	5268	191	316	286	266	261	236	231	286	221	301	186	436	206	456	286	388	6304	6244	6094	6374	6094	6565	5944	6194	6114	5959	6304	5919	6249	6560	6434	5969
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AA	7160	7300	6640	7035	7110	6790	6745	6710	6760	5704	6389	6249	6074	6785	6029	6214	371	231	171	266	311	246	306	316	206	226	296	271	186	341	301	263	6389	6004	5569	6224	6349	6389	6384	6104	6274	5654	6174	5959	6254	6164	6504	5669
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Figure 4. Uniform signal across Corning spheroid microplate. Representative image of color-scaled luminescence signal generated from an assay where DU 145 spheroids were exposed to a final concentration of buffer (green) or 1 µM staurosporine (red). Green corresponds to high luminescence values, high viability. Red corresponds to low luminescence values, low viability.

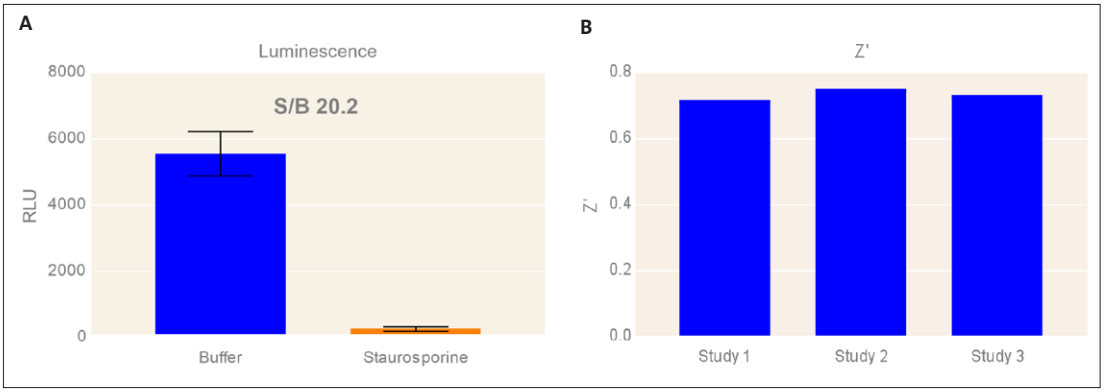

Figure 5. Large, robust luminescence assay window. (A) Mean ± SD luminescence of DU 145 spheroids exposed to buffer or 1 µM staurosporine, resulting in S/B of 20.2. $p<0.0001$ with unpaired t-test. (B) Excellent Z' values above 0.7 in 3 independent studies. N = 2304 wells in 3 independent studies.

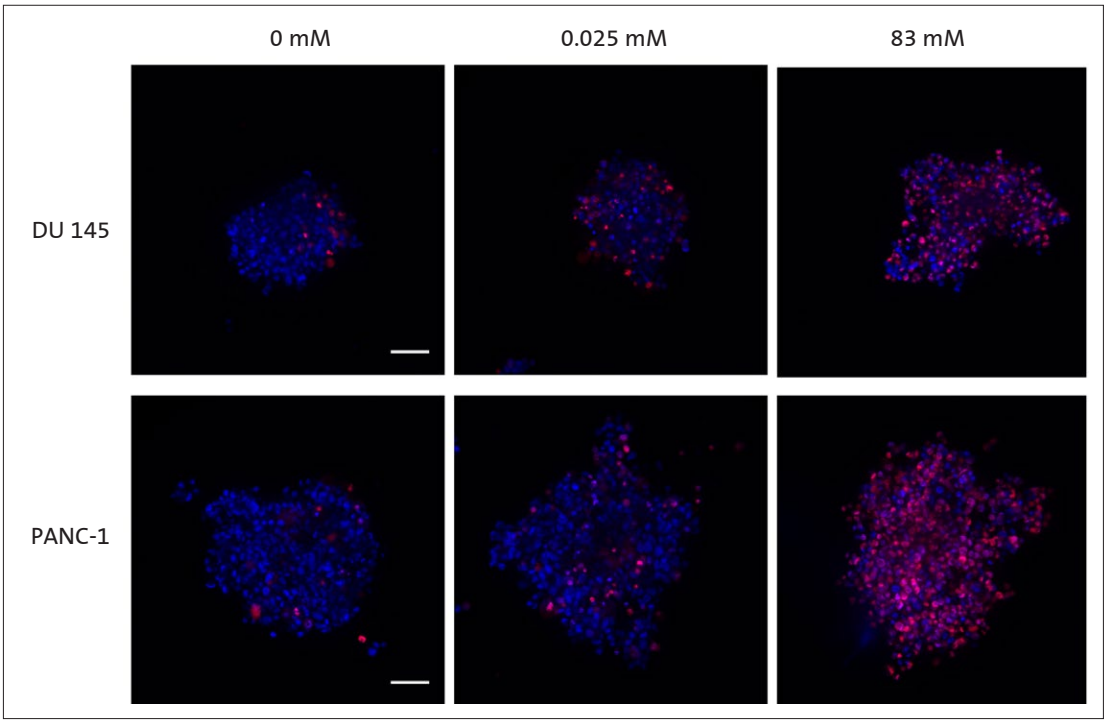

Figure 6. Confocal imaging of spheroids within Corning 1536-well spheroid microplate. Representative z-stack images of DU 145 (top) and PANC-1 (bottom) spheroids exposed to 0 mM, 0.025 mM, and 83 mM cisplatin (left, middle, right, respectively) for 24 hours. Spheroids were stained with Hoechst (blue) and PI (red) to assess cell viability. Spheroids were imaged directly in the spheroid microplate with the Thermo Fisher CellInsight™ CX7 high-content screening platform using a 10X objective. Scale bar = 100 µm.

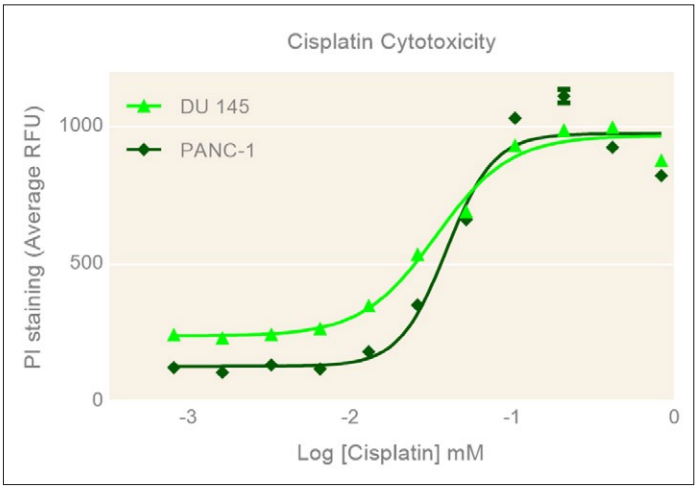

Figure 7. Concentration-dependent cisplatin cytotoxicity. Mean ± SD PI fluorescence of DU 145 and PANC-1 spheroids following 24 hours exposure to varying concentrations of cisplatin. N = 96 wells.

Conclusions

To screen large compound libraries against 3D cell models the proper tools are necessary to achieve quality results. The Corning® 1536-well spheroid microplate allows for the formation of uniform, single spheroids in each well that are ideally suited for high throughput 3D drug screens. The black sidewalls and clear bottom design of the Corning spheroid microplate make it ideally suited for imaging, luminescent, and fluorescent cell-based assays. Due to the small well volume of 1536-well microplates, accurate and consistent dispensing are essential. The INTEGRA VIAFILL and VIAFLO 384 instruments utilized for liquid handling for the 1536-well spheroid microplate result in assays with high signal-to-background ratios with excellent and consistent Z' values.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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