Ultra-High Throughput Production of Spheroids with AggreWell™

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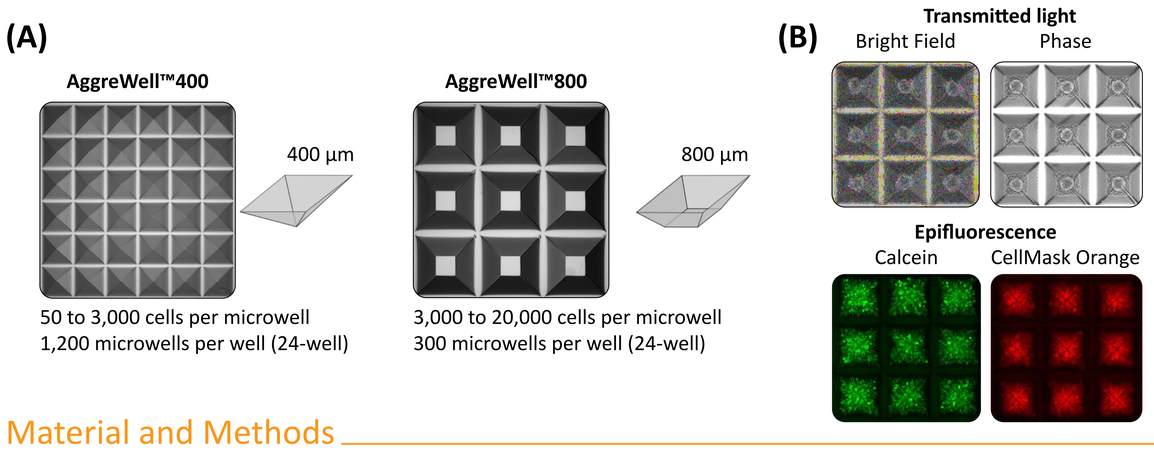
Introduction

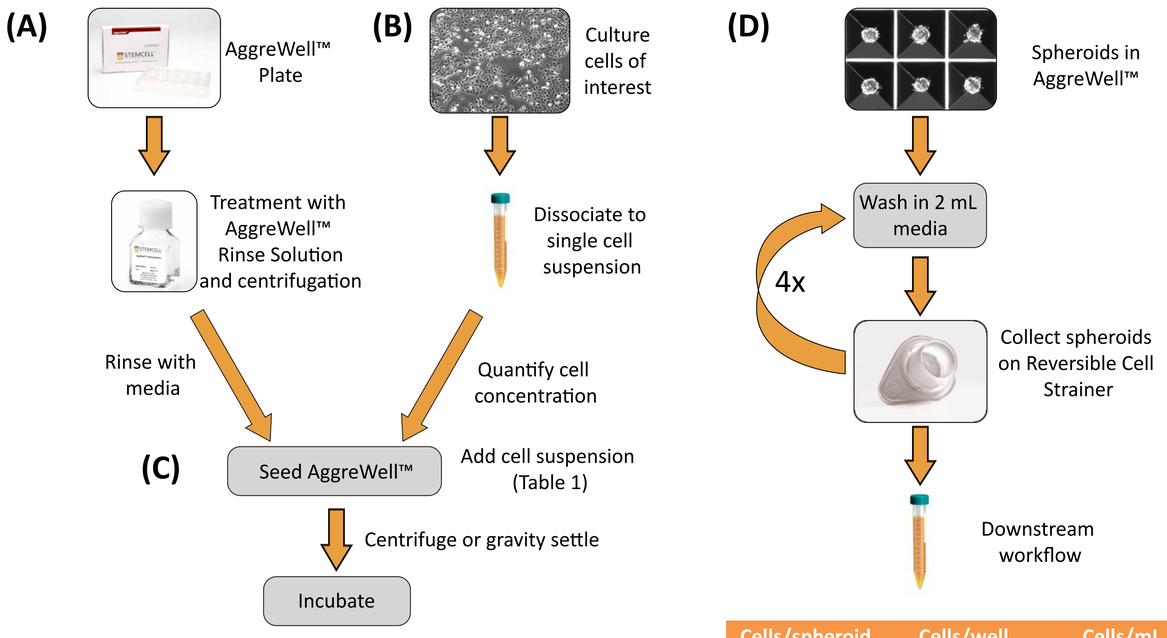
3D culture systems are increasingly being employed to better replicate the tumor microenvironment for cancer research, drug development, and toxicity screening. Many recent publications highlight the use of 3D culture methods as being more predictive than traditional 2D culture methods in determining drug efficacy in vivo[1-4]. However, 3D culture and cell aggregate or sphere generation techniques can be laborious, time consuming and low throughput. AggreWell™ plates have been widely used for 3D culture of embryoid bodies from human pluripotent stem cells, for neural and chondrogenic differentiation, and generation of cellular spheroids[5-7]. Herein we describe the use of new injection-molded AggreWell™ plates, available in multiple microwell sizes and formats (Figure 1), for ultra-high throughput tumor spheroid production and analysis. New AggreWell™ plates also have improved small molecule compatibility, optical characteristics, and consistent microwell alignment compatible with both automated and manual assays.

Figure 1. AggreWell™ plate formats. (A) AggreWell™ plates are available in 400 µm and 800 µm microwell sizes to accommodate spheroids of up to 3,000 or 20,000 cells, respectively. **(B)** New AggreWell™ plates are compatible with both transmitted and fluorescent microscopy techniques allowing for analysis of spheroid size and morphology without resuspension and separate imaging procedures.



Material and Methods

Figure 2. Spheroid production in AggreWell™. (A) To prepare AggreWell™ (STEMCELL Technologies Cat #27845) plates and remove air bubbles from the microwells, wells were treated with 500 µL of AggreWell™ Rinse Solution (STEMCELL Technologies, Cat #07010), centrifuged at 2,000g, and rinsed with 2 mL of DMEM. **(B)** For seeding AggreWell™ plates: Du145 prostate cancer, MCF7 breast cancer, and A549 lung cancer cells (ATCC) maintained in RPMI1640 (Du145 and MCF7 cells) or α-MEM (A549 cells) plus 10% Fetal Bovine Serum were trypsinized to a single cell suspension and quantified. **(C)** AggreWell™ plates were seeded with 2 mL of the appropriate concentration cell suspension (Table 1) and either centrifuged immediately (100g) or allowed to settle by gravity. AggreWell™ plates were stored and maintained at 37˚C and 5% CO_2 and analyzed at 1-2 days post-seeding via tiled microscopy imaging and image analysis via ImageJ (NIH, Bethesda). Data was analyzed using GraphPad Prism 5.0 software with size distributions plotted as frequency distributions, and spheroid morphology data calculated as follows: Estimated Volume (μm^3) = 4/3 *∏*(major axis/2*minor axis/2*average axis/2, and Compaction (cells/mm^3) = Estimated volume / input cell number. **(D)** For resuspension, AggreWell™ wells were treated with 4 successive washes of media and the resuspended spheroids collected on a 37 µm Reversible Cell Strainer (STEMCELL Technologies Cat #27215). Cell viability of dissociated Du145 and MCF7 spheroids was measured via AO/DAPI staining and analysis on a NC250 Nucleocounter (ChemoMetec).



Cells/spheroid	Cells/well	Cells/mL
100	1.2×10^5	6.0×10^4
200	2.4×10^5	1.2×10^5
500	6.0×10^5	3.0×10^5
1,000	1.2×10^6	6.0×10^5

Table 1: Seeding densities for AggreWell™400 generated spheroids. Sphere size can be controlled in AggreWell™ plates by adjusting the seeding density of cells in each well to yield the desired cell number per microwell.

References

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[3]Ghosh et al. J Cell Physiol, 204: 522-31, 2005.
[4]Ernst et al. Clin Cancer Res, 15:6541-50, 2009.
[5]Ungrin et al. PLoS ONE e1565, 2008.
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[7]Xia et al. J Mol Neurosci 51(2):237-48. 2012.

Results

Figure 3. AggreWell™ generated cancer spheroids. (A) Du145, MCF7 and A549 cancer cells seeded into AggreWell™ readily form one spheroid per microwell within 24-48 hours. Spheroid morphology varies between cell lines and correlates, in part, with 2D culture morphology. (B) In the 24-well plate of AggreWell™400, 1,200 spheroids can be easily generated per well during a single experiment.

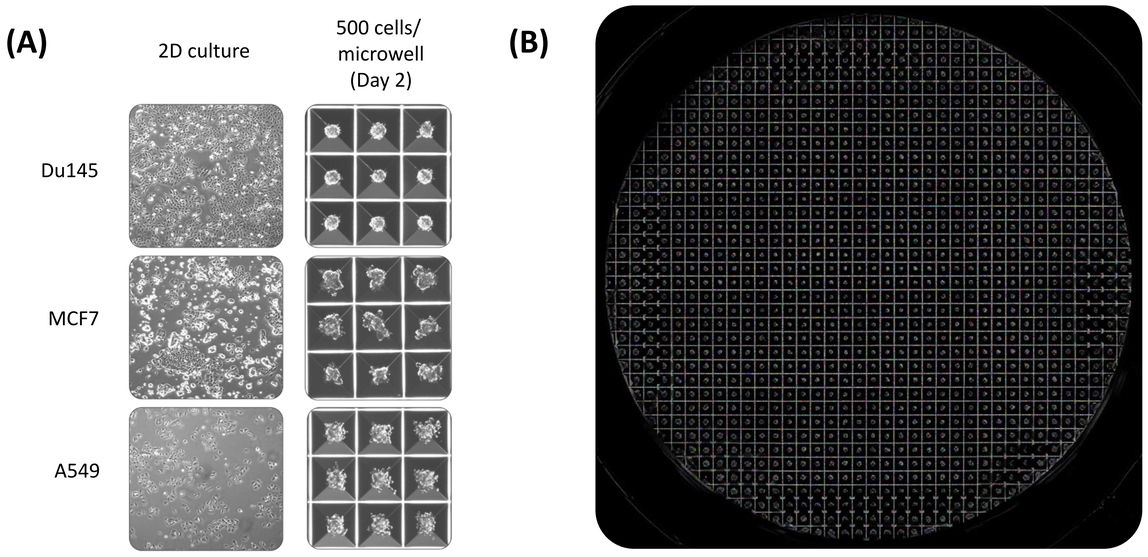
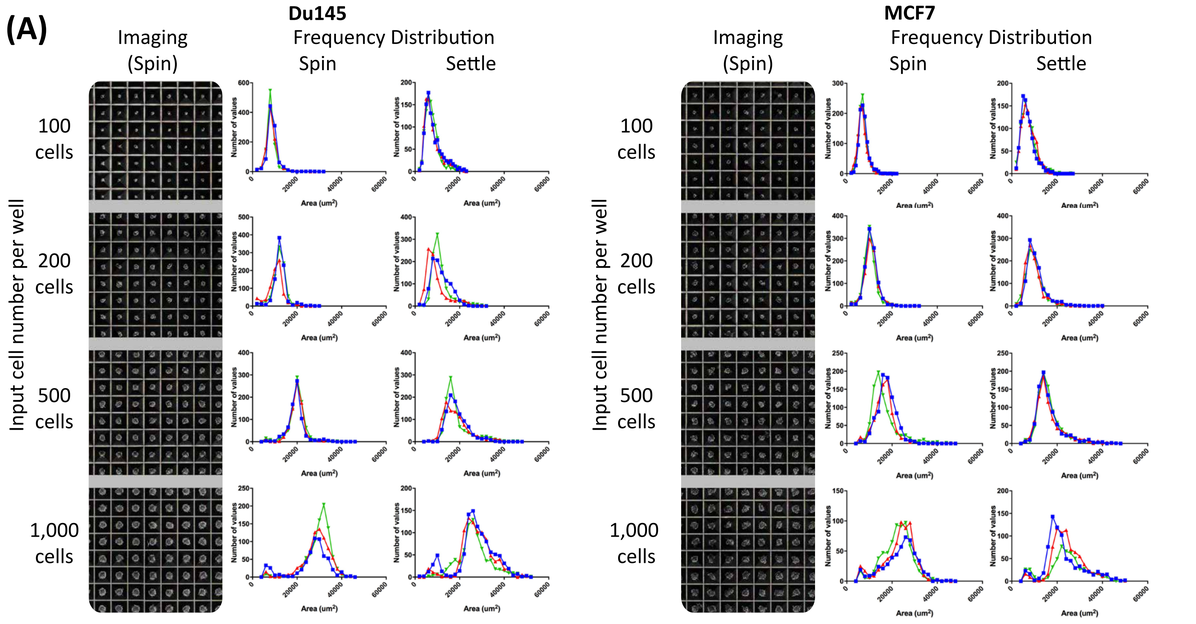


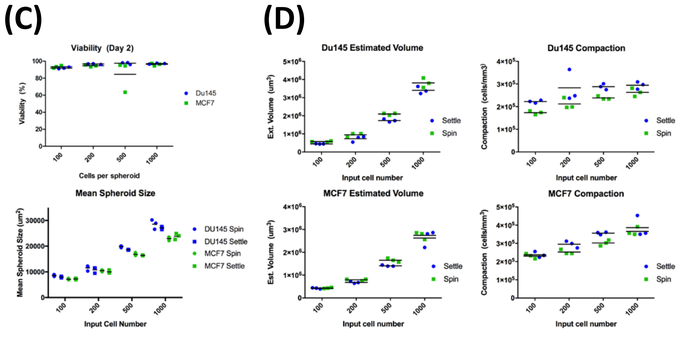
Figure 4: AggreWell™ spheroid sizing. (A) Input cell number determines spheroids size after 24-48 hours for both Du145 and MCF7 cells. Frequency plots show a tight distribution for spheroid projected area with a low variance between replicate wells for each seeding density. **(B)** Descriptive statistics for spheroid sizing frequency distributions. **(C)** Viability after spheroid dissociation is greater than 90% for all conditions (one outlier). Mean spheroid size varies slightly between Spin vs Settle and Du145 vs MCF7 conditions. **(D)** Estimated volume and cell compaction increase with input cell number for both Du145 and MCF7 cells.



(B)

Du145	Spin				No Spin			
Input cell	100	200	500	1000	100	200	500	1000
25% Percentile	7409.5	10202.5	18251.1	26525.0	5430.6	7828.5	14842.9	23475.2
Median	8396.8	11753.8	19910.0	29661.9	6993.9	9670.0	17280.0	26876.6
75% Percentile	9432.7	13034.7	21309.4	32273.1	9536.9	12494.5	20711.3	31223.5
Mean	8602.3	11522.0	19834.8	28562.8	7986.7	10805.3	18610.8	27097.1
Std. Deviation	2409.7	3115.6	3860.7	6404.5	3652.7	4397.1	5561.8	7514.8
Lower 95% CI of mean	8451.1	11313.9	19563.1	28089.0	7762.7	10526.3	18259.2	26598.8
Upper 95% CI of mean	8753.5	11730.0	20106.6	29036.5	8210.6	11084.3	18962.5	27595.5

MCF7	No Spin				No Spin			
Input cell	100	200	500	1000	100	200	500	1000
25% Percentile	5877.8	8942.9	14027.9	19157.3	5097.8	7477.8	12823.0	19511.5
Median	6898.3	10373.1	16283.3	23987.2	6717.4	9265.5	14900.9	22797.7
75% Percentile	8088.7	11845.1	18880.0	27508.9	8913.9	11671.0	18333.9	27979.1
Mean	7128.1	10473.8	16831.4	22946.4	7244.4	9981.4	16411.1	23854.9
Std. Deviation	1996.0	2794.3	4778.7	6725.2	3016.3	3705.0	5789.5	7925.2
Lower 95% CI of mean	7003.3	10294.5	16500.8	22400.8	7059.4	9747.8	16009.4	23256.9
Upper 95% CI of mean	7252.9	10653.0	17162.0	23492.0	7429.4	10215.0	16812.8	24452.8



Conclusions

AggreWell™ is a tool for the rapid generation of uniform cancer spheroids for ultra high throughput workflows. Spheroids can be generated via centrifugation for higher uniformity, or via gravity settling for high throughput or robotic scale up. Spheroid formation in AggreWell™ is reproducible between replicate wells. Use of AggreWell™ for tumor spheroid generation allowed for high-throughput production of > 28,000 spheroids per plate, well beyond the scale possible with other methods including 96- or 384-well V-bottom or hanging drop methods. Spheroids generated in AggreWell™ are highly uniform, display cell type specific morphology, and are compatible with existing downstream assays, enabling high throughput and physiologically relevant 3D culture.