Drug Screening and Phenotypic Analysis in a Microwell-based 3D Cell Culture System

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Introduction

Increasingly, the cancer research field is recognizing the need to transition to more physiologically relevant 3D screening systems that better replicate the cell-cell interactions, microenvironment and mass transfer properties of *in vivo* tumors. 3D systems have been shown in several studies to be more predictive of drug efficacy than traditional culture methods[1,2]. However, with improved physiological relevance comes increased heterogeneity that can complicate cell culture practices and confound standard assay readouts. Furthermore, many 3D culture systems are laborious, time-consuming and low throughput. AggreWell™ plates are microwell-based 3D cell culture devices that produce many size-controlled cancer spheroids per well from a single pipetting step (Figure 1). Within each imaged well, every spheroid can be individually identified, indexed, and analyzed to provide multiple repeated measures per treatment condition to yield more representative and predictive data.

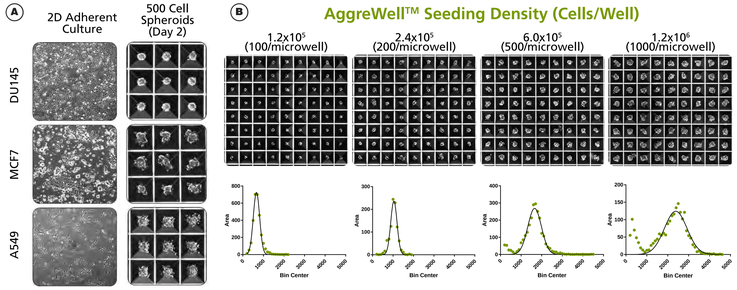

Figure 1: AggreWell™ Supports Uniform and Size-Controlled Production of Spheroids. (A) 3D spheroid morphology in AggreWell™ varies by cell line and is related to the morphology of the starting 2D culture. (B) In AggreWell™, spheroid size can be controlled by varying the input seeding volume, producing tight distributions of aggregate sizes.

Methods

Herein, we document the use of AggreWell™ to perform a 3D drug screening study using cancer spheroids of the MCF7 breast cancer cell line. Briefly, 500 cell cancer spheroids were made by seeding 6x10^5 cells in AggreWell™400 24-well plates (Cat #34411) in serum-free MammoCult™ (Cat #05620) media. 24 hours after seeding, spheroids were exposed to drug treatments via 50% media exchange. Drug compounds were selected to target the luminal, basal, and milk epithelial cell lineages present in breast tissue (Figure 2A) to assess the sensitivity of MCF7 cells to these treatments. EC50 ranges for each drug were determined for concentration ranges up to 100 µM and 20 µM for Tamoxifen (TMX) and Lapatinib (LTB) respectively. DAPT up to concentrations of 200 µM had no effect beyond that of vehicle. For multi drug treatments, concentrations of 20 µM, 5 µM and 10 µM were used for TMX, LTB, and DAPT respectively. Treated spheroids were imaged, dissociated and quantified for cell viability via AO/DAPI staining and analysis in a NC-250 Nucleocounter (ChemoMetec). ImageJ analysis was performed to measure morphological parameters for each of 16 spheroids per well. The resulting data set was interrogated using Principle Component Analysis (PCA) to identify parameters predictive of drug-treatment effects and graphed to identify combinations that permit treatment classification.

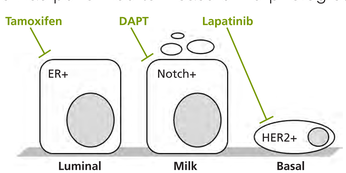

Figure 2: Breast Cancer Epithelial Cell Lineages and the Drugs That Target Them.

Results

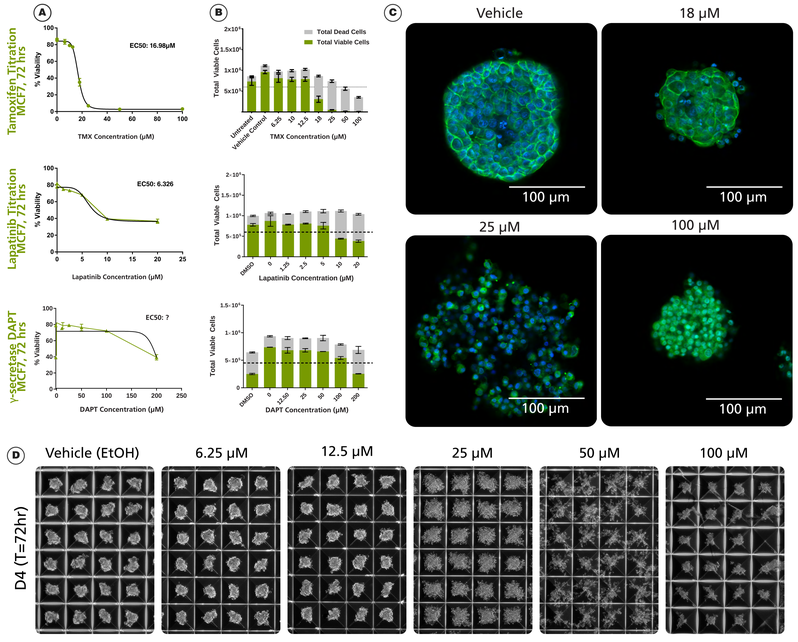

Figure 3: EC50 Determination and Corresponding Morphological Changes in MCF7 Spheriods. TMX, which targets the luminal cells most common in the MCF7 cell line, has the greatest impact on cell viability and morphology while LTB and DAPT have moderate or no effect based upon **(A)** viability-based EC50 curves, or **(B)** comparison cell viability in treated spheroids. **(C)** Dose-dependent changes in E-Cadherin expression (green) indicates alteration of morphology at cellular level. **(D)** Representative images of TMX-treated spheroids in AggreWell™ plates. Doses near the EC50 concentrations exhibit spheroid disaggregation, while higher doses show no morphology change due to rapid cell death.

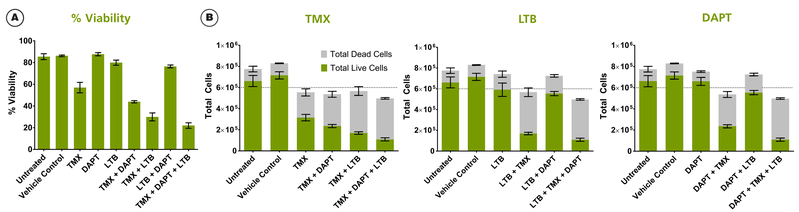

Figure 4: Synergistic Effects of Multi Drug Treatments in MCF7 Spheroids. LTB and DAPT show little effect alone, but demonstrate synergistic reductions of cell viability when treated in combination with TMX. 3-drug treatment displays further viability reductions. As DAPT has no effect in single drug treatment, this suggests that co-treatment sensitizes some portion of the population to DAPT. Data presented as **(A)** percent viability and **(B)** total viable and dead cells.

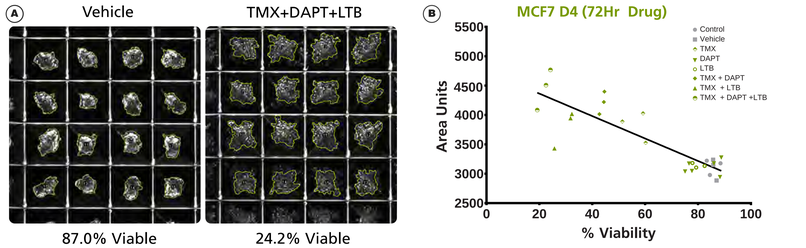

Figure 5: Correlation of % Viable Cells and Spheroid Morphology. (A) Representative images show clear difference in spheroid size and morphology between vehicle-treated spheroids (87.0% viable) and 3-drug treated (24.2% viable) cultures. **(B)** Control and vehicle-treated spheroids display tight clustering around 80% viability and low spheroid area. Drug-treated spheroids show reduced viability correlating with spheroid disaggregation resulting in increased measure area.

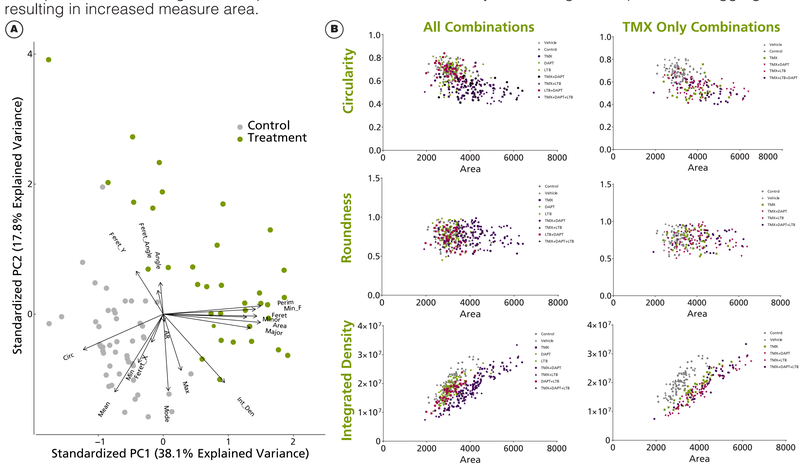

Figure 6: Identification of Phenotypic Markers of Drug Treatment. (A) PCA analysis shows non-overlapping clustering of control and 3-drug treatment conditions with Eigenvectors highlighting the features most strongly associated with each treatment. These parameters can therefore be used to better define the decision plane between treatment conditions for testing of future unknown compounds. **(B)** Selected plots of pairwise comparisons of parameters highlighted in A allow for visualization of population separation by treatment group.

Summary

AggreWell™ is a microwell based cell culture system that:

- Is an efficient tool for generating 3D spheroids
- Provides a standardized method for performing 3D drug screens
- Provides multiple individually identifiable and measurable spheroids per treatment condition
- Allows determination of EC50 or IC50 curves via cell-based assays including viability

References:

[1]Gangadhara et al. BMC Cancer, 16(1):345, 2016.
[2]Nath et al. Pharmacol Ther 163:93-108, 2016.