Cancer Therapeutic Screening in Tumor Spheroids Co-cultured with Endothelial Cells using Corning® Spheroid Microplates

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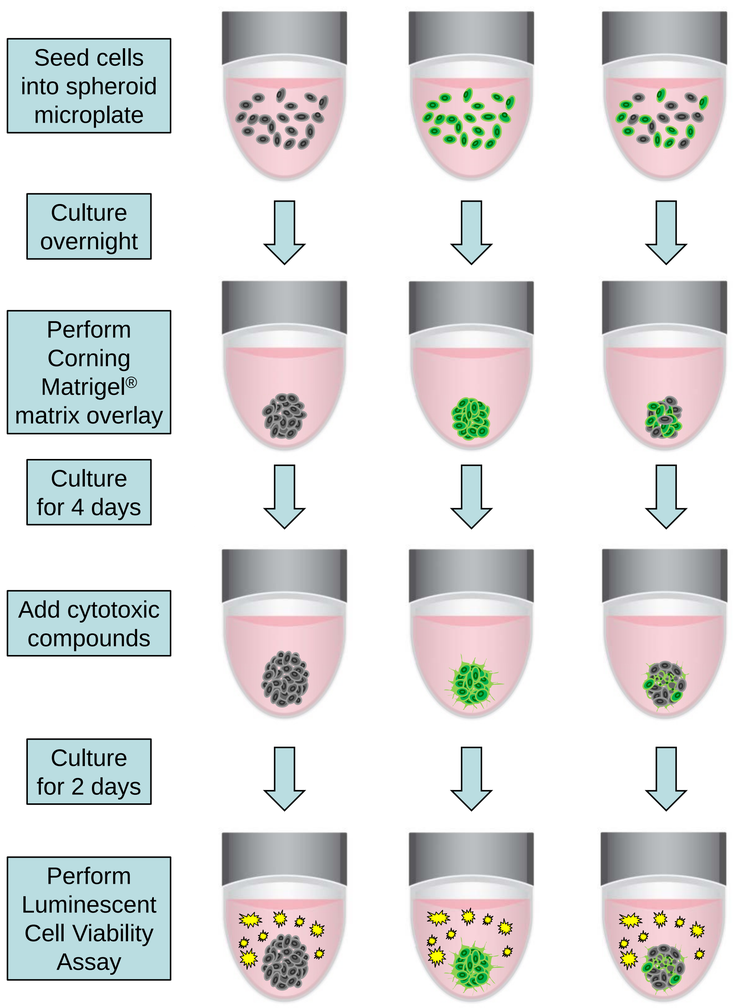
ABSTRACT

Two-dimensional (2D) cell culture models have historically been used in drug discovery for the development of cancer therapeutics due to their ease of use and established compatibility with high throughput screening. Recently, more elaborate, three-dimensional (3D) cell culture models have been developed, which better mimic the *in vivo* tumor microenvironment. These 3D models bridge the gap between successful *in vitro* studies and success in clinical trials. For example, 3D cultures develop hypoxic cores and demonstrate gradients of various soluble factors and a diffusion profile for drugs similar to tumors. Understanding the complex interactions between cancer cells and other cell types in the tumor microenvironment, such as fibroblasts, endothelial cells, and immune cells, is also critical to predicting therapeutic efficacy. This is important as many factors, such as the secretion of cytokines and extracellular matrices may also impact drug resistance and sensitization. However, conventional methods for 3D cell culture are often time consuming, display increased variability and lack throughput options. Corning® spheroid microplates are multiple well, cell culture plates with opaque walls and unique clear, round well-bottom geometry that utilize Corning Ultra-Low Attachment surface coating. The coating is hydrophilic, biologically inert, and non-degradable, which enables the rapid and highly reproducible formation of a single multicellular tumor spheroid, centered in each well. In this study, an epithelial tumor cell line was cultured in 96-well Corning spheroid microplates with and without the presence of GFP-expressing mouse endothelial C166 cells. Tumor spheroid viability was screened after treatment with various chemotherapeutics in both single tumor cell line culture and endothelial co-culture conditions. Dose-dependent responses of selected chemotherapeutics were compared, demonstrating the importance of including multiple cell types in 3D assays to more closely mimic the *in vivo* tumor microenvironment.

Corning Spheroid Microplates

Corning 96- or 384-well cell culture spheroid microplates with round well-bottom geometry and Corning Ultra-Low Attachment surface coating enabling the rapid and highly reproducible formation of a single multicellular tumor spheroid, centered in each well. Clear bottom and opaque walls support spheroid visualization and luminescent and fluorescent assays directly in the culture plate.

Assay Overview



METHODS

Spheroid Formation and Imaging

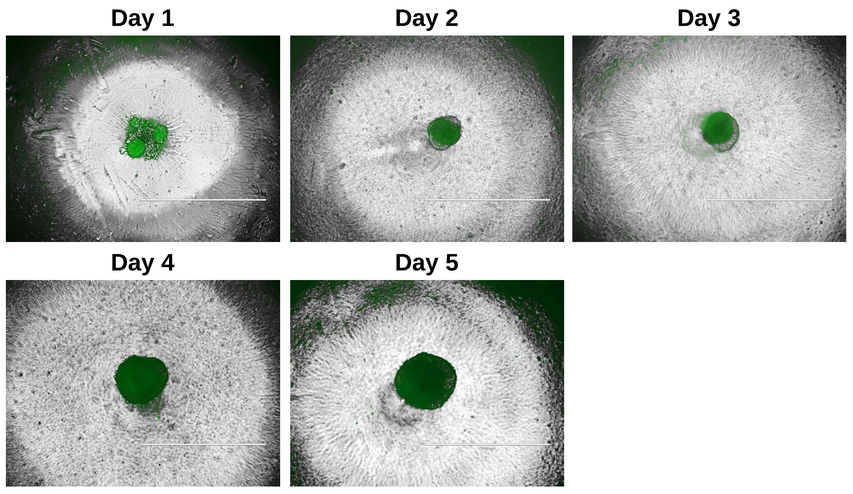
1. HT-29 (ATCC® Cat. No. HTB-38) and C166-GFP (ATCC Cat. No. CRL-2583) were cultured in 2D format following ATCC's protocols.
2. Confluent cells were harvested and seeded into 96-well spheroid microplates (Corning Cat. No. 4520) at 2,000 total cells per well either in mono-culture or in co-culture with a seeding ratio of 1:1. Cells were seeded in 100 µL per well of culture medium [IMDM (Corning Cat. No. 10-016-CM) supplemented with 10% FBS (Corning Cat. No. 35-010-CV)]. Cells were cultured in a humidified 37˚C, 5% CO_2 incubator for 24 hours for the formation of a spheroid in each well.
3. After 24 hours, the spheroids were overlaid with 100 µL per well of 1.76 mg/mL Corning Matrigel matrix (Corning Cat. No. 354234) diluted in culture medium. The spheroid microplates were centrifuged at 220 x g at 4˚C for 2 minutes and returned to a humidified 37˚C, 5% CO_2 incubator for 4 days. Images were taken daily using an EVOS™ microscope with transmitted light and GFP channels.
4. Five days after cell seeding, spheroids were imaged using a Thermo Fisher CellInsight™ CX7 High-Content Screening platform.

Cell Viability Assays

1. Five days after cell seeding, 50 µL per well of compounds in culture medium containing 0.5% dimethyl sulfoxide [DMSO] (Corning Cat. No. 25-950-CQC) were added. 50 µL of culture medium containing 0.5% DMSO was used as vehicle control.
2. Spheroids were cultured for an additional 48 hours prior to cell viability assays.
3. For cell viability assays, cells were assayed in the spheroid microplate using CellTiter-Glo® 3D (Promega Cat. No. G9683) following vendor's protocols. Luminescence was detected using a PerkinElmer EnVision™ Multilabel plate reader.

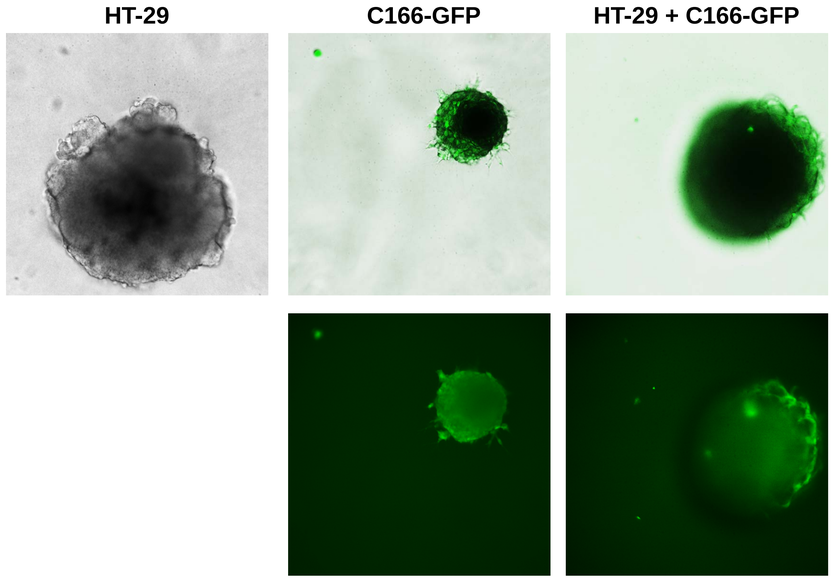
Spheroid Formation

Co-culture Formation



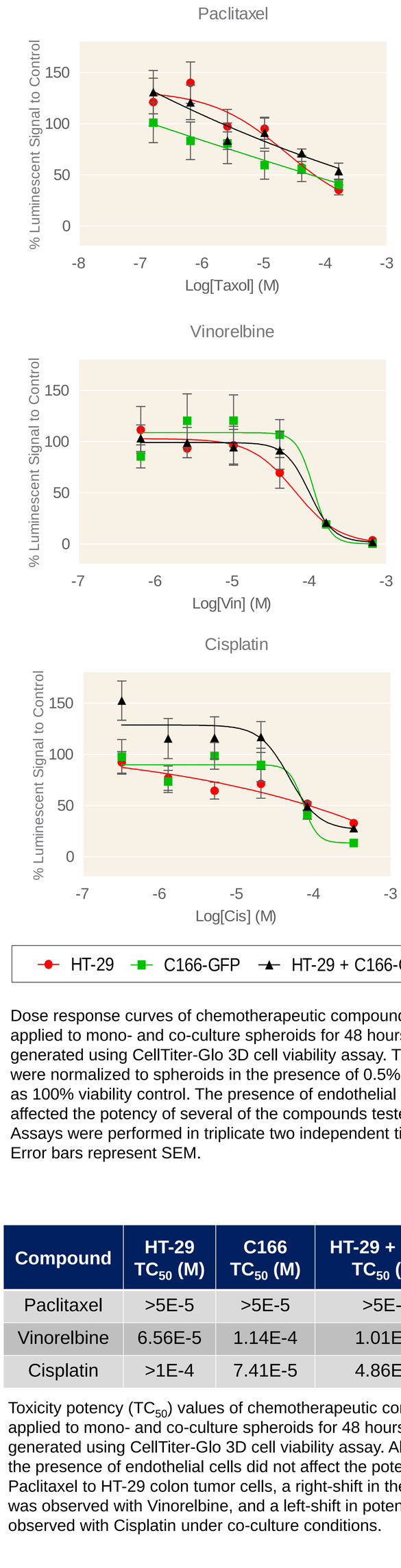
Formation of co-culture spheroids. C166-GFP (green) and HT-29 cells with a Corning Matrigel matrix overlay formed spheroids over 5 days. Images taken with an EVOS microscope with 4X objective with transmitted light and GFP channels.

Cell Viability Assay Spheroids



Images of mono- and co-culture spheroids. HT-29 and C166-GFP endothelial cells were seeded at a total of 2K cells/well in mono-culture and in co-culture at a ratio of 1:1. Twenty-four hours after cell seeding, spheroids were overlaid with Corning Matrigel matrix and cultured in the Corning spheroid microplate for 96 hours. Images were obtained using a Thermo Fisher CellInsight CX7 in brightfield and confocal modes with 10X objective. Top row: Brightfield images using 10X objective (HT-29), or brightfield images overlaid with GFP channel composite images taken from ten individual slices at 15 µm step size in confocal mode (C166-GFP containing spheroids). Bottom row: Single slice from center of spheroid in confocal mode using GFP channel (C166-GFP containing spheroids only).

Mono- and Co-culture Responses to Chemotherapeutic Compounds



Dose response curves of chemotherapeutic compounds applied to mono- and co-culture spheroids for 48 hours were generated using CellTiter-Glo 3D cell viability assay. The data were normalized to spheroids in the presence of 0.5% DMSO as 100% viability control. The presence of endothelial cells affected the potency of several of the compounds tested. Assays were performed in triplicate two independent times. Error bars represent SEM.

Compound	HT-29 TC_{50} (M)	C166 TC_{50} (M)	HT-29 + C166 TC_{50} (M)
Paclitaxel	>5E-5	>5E-5	>5E-5
Vinorelbine	6.56E-5	1.14E-4	1.01E-4
Cisplatin	>1E-4	7.41E-5	4.86E-5

Toxicity potency (TC_{50}) values of chemotherapeutic compounds applied to mono- and co-culture spheroids for 48 hours were generated using CellTiter-Glo 3D cell viability assay. Although the presence of endothelial cells did not affect the potency of Paclitaxel to HT-29 colon tumor cells, a right-shift in the potency was observed with Vinorelbine, and a left-shift in potency was observed with Cisplatin under co-culture conditions.

CONCLUSIONS

- The Corning 96-well spheroid microplate can be used to generate and perform viability assays with co-culture spheroids containing a network of tubule-forming endothelial cells in an easy-to-use, high throughput format.
- The presence of endothelial cells with tumor cells in a spheroid affects the potency of several chemotherapeutic compounds.

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