Establishing a Reproducible Pipeline for High-Throughput Screening of 3D Tumor Spheroids

Nikta Ziaei[1], Martin Ondra[1, 2], Marian Hajduch[1, 2]

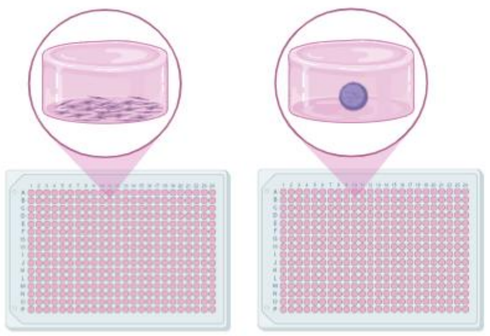
[1]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc

[2]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Czech Advanced Technology and Research Institute, Palacky University Olomouc

BACKGROUND

Traditional two-dimensional (2D) cell cultures fail to replicate the complex architecture and microenvironment of solid tumors. In contrast, three-dimensional (3D) systems, like tumor spheroids, provide a more physiologically relevant model by better mimicking cell–cell and cell–matrix interactions. These models enable more accurate studies of tumor behavior, drug penetration, and treatment response.

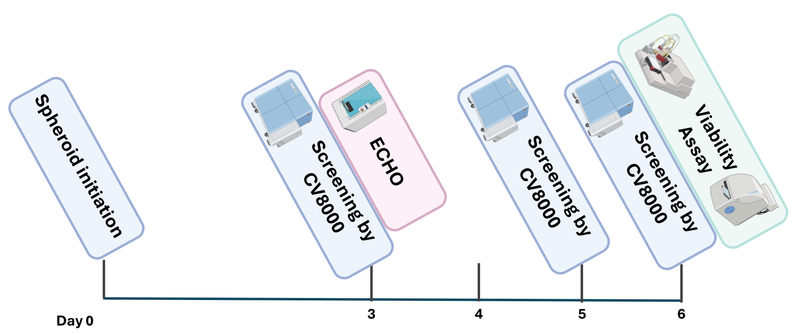
This study established an optimized high-throughput screening (HTS) workflow using HCT116 spheroids in 384-well plates to enhance preclinical drug screening. The workflow integrates automated formation, ECHO compound dispensing, CV8000 high-content imaging, and MTT viability assays to quantify cytotoxic effects. This integrated approach offers a streamlined, reproducible, and biologically relevant platform for evaluating anticancer compounds in a 3D context.



METHODS

Workflow Optimization

- HCT116 spheroid formation was transitioned from liquid overlay (agarose-coated plates) to 384-well, U-bottom, low-attachment plates compatible with HTS and Echo dispensing. Culture conditions were optimized for consistent size and morphology.
- Compounds were dispensed at multiple concentrations with high precision using the Echo 650 acoustic liquid handler.
- Spheroid growth and morphology were monitored using the CellVoyager CV8000 High-Content Screening System, and automated image analysis quantified size and shape.
- Cytotoxicity readouts were optimized by transitioning from CellTiter-Glo to the MTT assay for improved compatibility and reproducibility with the 3D model.
- Assay data were normalized and analyzed to generate dose–response curves and quantify compound-induced cytotoxicity.



CONCOLUSION

We successfully established a fully optimized and highly reproducible 3D HTS platform using HCT116 spheroids. This integrated and robust workflow (ECHO, CV8000, MTT) enhances the predictive power of drug screening models. Highly scalable and ready for application, the platform will now be used for the main screening campaign against the LOPAC library to identify novel cytotoxic compounds in a clinically relevant 3D context.

AKNOWLEDGMENT

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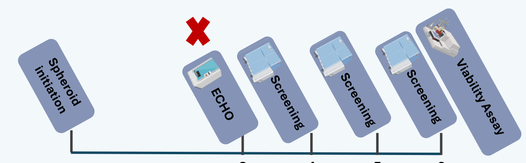
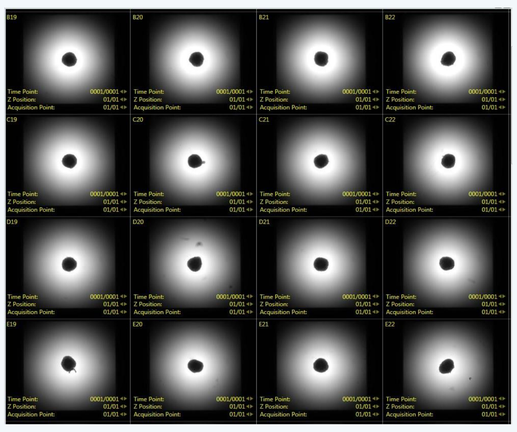
RESULTS

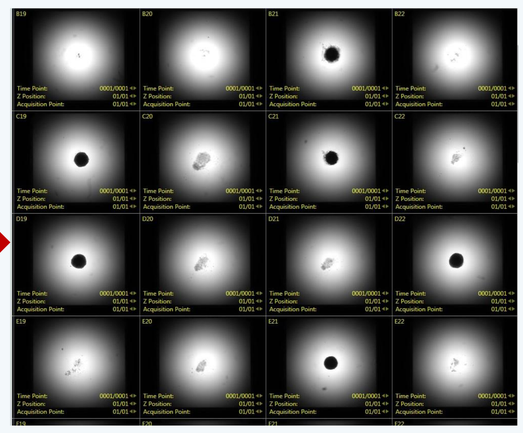
Agarose-coated plate



Before ECHO

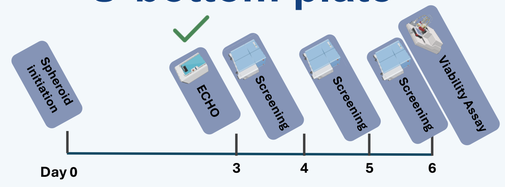


After ECHO

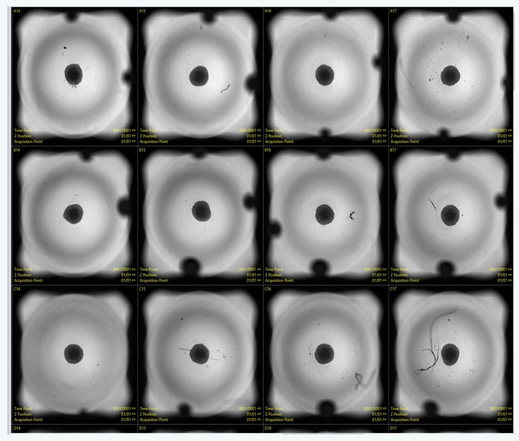


Instability of Spheroids in Agarose-Coated Plates Following Acoustic Dispensing. Spheroids formed using the liquid overlay technique in agarose-coated plates (Left: Before ECHO dispensing) **disintegrated** and lost structural integrity immediately following compound addition by the ECHO (Right: After ECHO dispensing). This result necessitated the optimization and transition to low-attachment U-bottom plates for the HTS workflow.

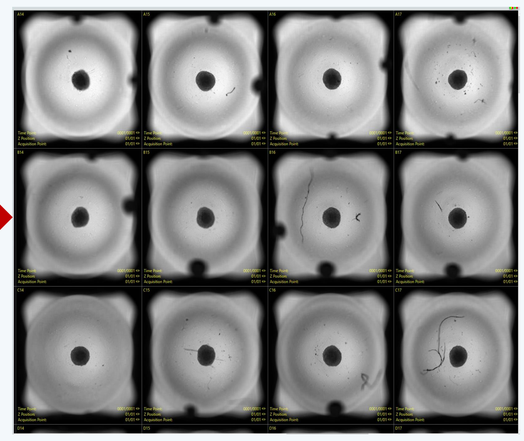
U-bottom plate



Before ECHO



After ECHO

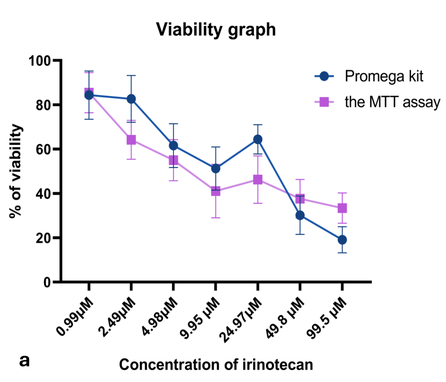


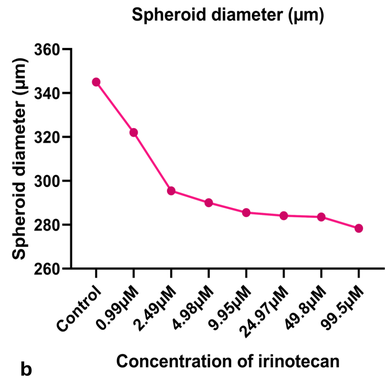
Stability of HCT116 Spheroids in U-bottom Low-Attachment Plates after Acoustic Dispensing. Following optimization, spheroids were formed in U-bottom, low-attachment plates. The images confirm that spheroids maintained excellent structural integrity and position (Right: After ECHO dispensing) compared to the pre-dispensing control (Left: Before ECHO), demonstrating the robustness of the optimized HTS protocol for acoustic liquid handling.

MTT assay optimization

	First biological replicate		Second biological replicate		Third biological replicate	
IC50 Irinotecan	5H incubation	7H incubation	5H incubation	7H incubation	5H incubation	7H incubation
0.5 mg/ml MTT	**11.8 µM**	37.2 µM	**10.9 µM**	21.2 µM	**11.59 µM**	28.8 µM
0.6 mg/ml MTT	12.2 µM	27.5 µM	14.6 µM	30.8 µM	13.8 µM	31.5 µM
0.7 mg/ml MTT	20.4 µM	58.5 µM	18.76 µM	96.8 µM	27.91µM	62.28 µM
PROMEGA KIT	**3.42 µM**		**11.32 µM**		**10.68 µM**	

Optimization of the MTT Viability Assay Readout Parameters. The MTT assay was optimized by testing three different final concentrations (0.5, 0.6, and 0.7 mg/mL) and two incubation times (5H and 7H) against the reference compound Irinotecan across three independent biological replicates. IC50 values derived from the optimized MTT conditions (0.5 mg/mL, 5H incubation) showed lower variability between replicates compared to the higher concentrations and longer incubation times and provided results comparable to the initial reference assay (Promega Cell Titer-Glo). Based on these results, 0.5 mg/mL MTT with a 5-hour incubation was selected for the final HTS protocol.





Viability Assay Comparison and Morphological Response to Irinotecan Treatment.
(a) Viability Graph: Dose-response curves for Irinotecan (0.99 µM to 99.5 µM) in HCT116 spheroids comparing the **optimized MTT assay** (purple) vs. Promega CellTiter-Glo (blue). Both assays confirmed dose-dependent cytotoxicity, validating the optimized MTT protocol.
(b) Spheroid Diameter (µm): Irinotecan treatment resulted in a dose-dependent reduction in spheroid diameter, confirming that high-content image analysis is a robust secondary endpoint for efficacy.