High throughput characterization of advanced 3D liver models for in vitro hepatotoxicity studies

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THE ADVANCED CELL MODEL COMPANY

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Introduction

Hepatotoxicity or drug-induced liver injury (DILI) continues to be a major cause for novel drug withdrawal from clinical trials. Recently, the use of three-dimensional (3D) *in vitro* models for drug testing has seen results more congruent to the *in vivo* response, enabling potential early detection of hepatotoxic therapeutics. However, the development and adoption of advanced cell models for cytotoxicity studies has been met with several challenges, such as being low-throughput, requiring manual handling, co-culture consistency and incompatibilities with existing 2D workflows.

Here we describe the use of advanced cell models comprised of liver cell lines (PH5CH8 and LX-2 cells) in 384-well format created with the RASTRUM platform. Cell health and viability in response to exposure to known hepatotoxic drugs were assessed using high content imaging in conjunction with live/dead viability dyes, as well as by measurement of ATP production and morphological analysis.

Workflow

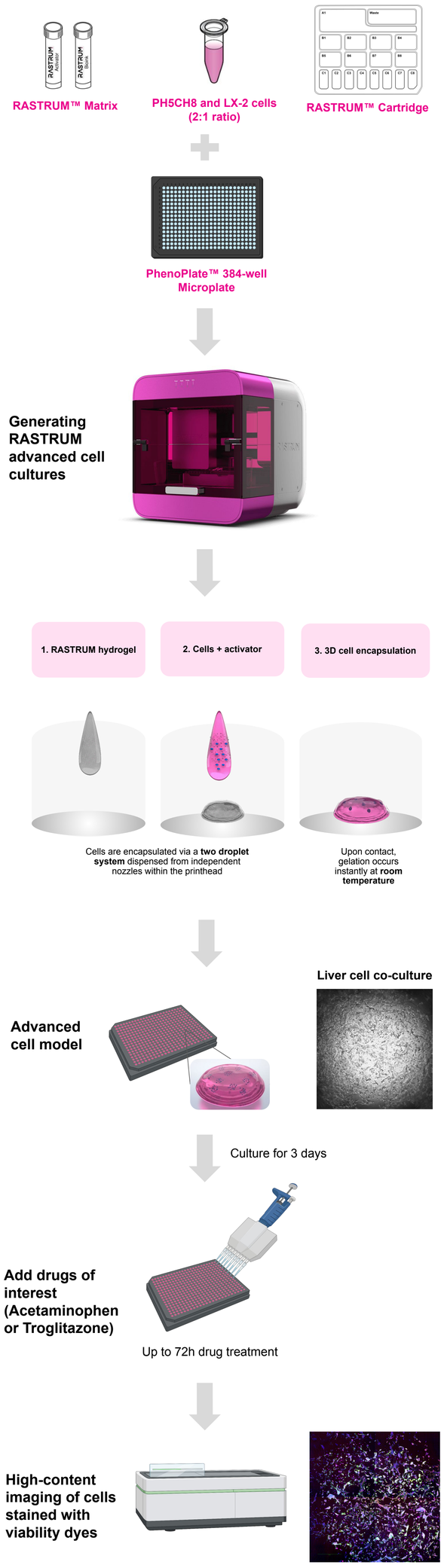


Fig. 1. The RASTRUM platform enables a simple workflow for creation of advanced 3D liver cell models in high-throughput formats, seamlessly compatible with existing drug screening methodologies and downstream analyses such as high-content imaging and end-point assays. Created with Biorender.com.

Results

Result 1: Highly consistent and reproducible 3D cell cultures containing PH5CH8 and LX-2 cells were established using RASTRUM. The liver tuned advanced matrix, including tissue-relevant adhesion peptides and proteins, enabled the co-culture of two essential cell types.

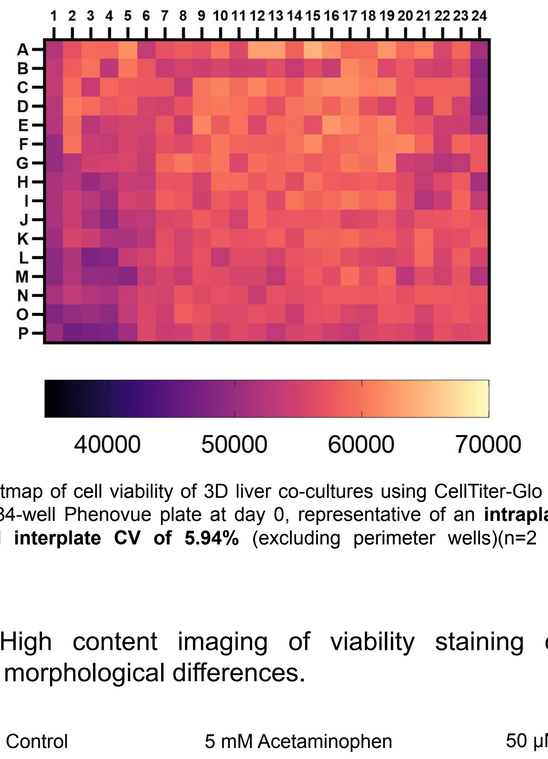


Fig. 2. Heatmap of cell viability of 3D liver co-cultures using CellTiter-Glo 3D assay across a 384-well Phenovue plate at day 0, representative of an **intraplate CV of 5.53% and interplate CV of 5.94%** (excluding perimeter wells)(n=2 biological replicates).

Result 2: High content imaging of viability staining clearly showed drug-related morphological differences.

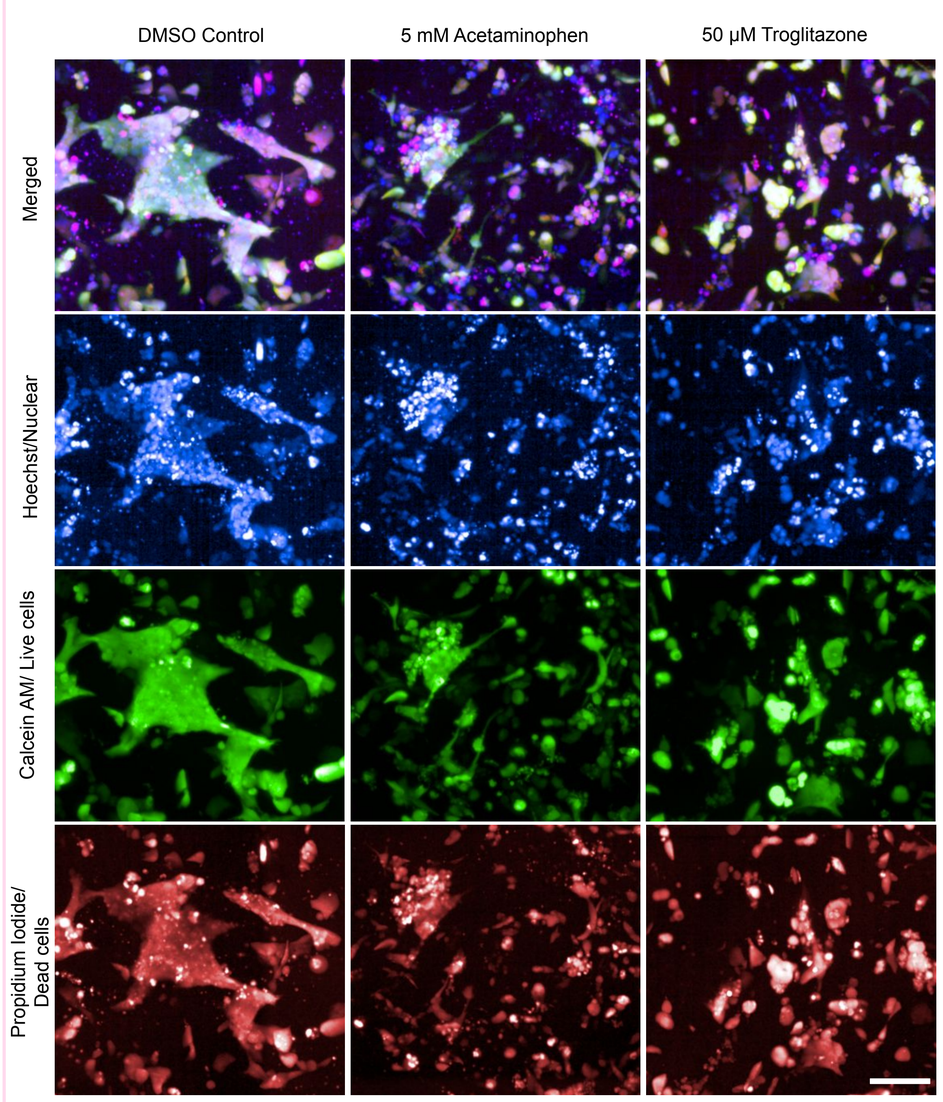
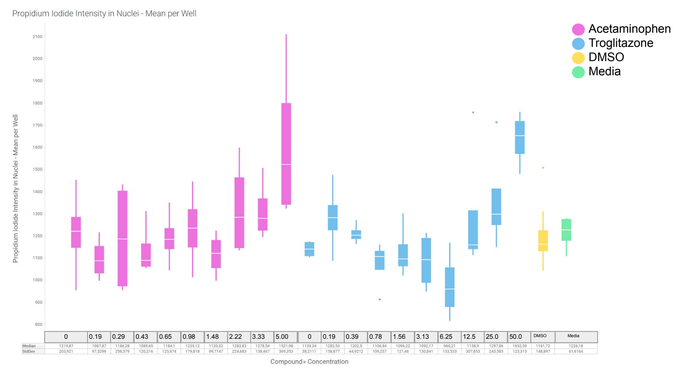


Fig. 3. Representative ROI (region of interest) of viability staining images of RASTRUM printed 3D liver co-cultures. Image stacks of 15 planes at a distance of 35 µm were acquired on an Opera Phenix™ high-content screening system in confocal mode using a 10x objective. Per well 4 fields were acquired. Of these a montage of the maximum intensity projection was created and analysed using Harmony® image analysis software. The printed cells were treated with Acetaminophen and Troglitazone at different concentrations. First column is related to DMSO control and second and third columns are correlated to increased concentrations of each drug. Scale bar = 200 µm.

Result 3: Cells were stained with PhenoVue™ Live/Dead Cell Viability Assay Kit to detect compound induced cell death. Image analysis was done using Harmony® high-content imaging and analysis software. To analyze the effect of the compounds the intensity of Propidium Iodide (PI) in the nucleus and morphological changes within the cell area were analyzed. To analyze morphological changes, SER and Haralick texture properties were extracted. SER texture (Spots, Edges and Ridges) quantifies the occurrence of eight characteristic intensity patterns such as spots, edges and ridges within the image. Haralick texture analysis is based on a co-occurrence matrix and allows, for example, the quantification of homogeneity within an image.

i.



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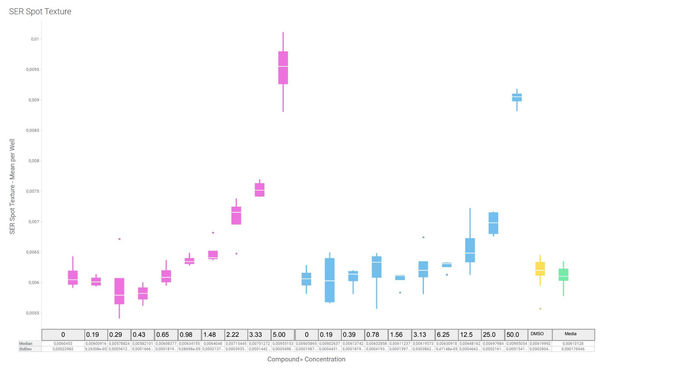

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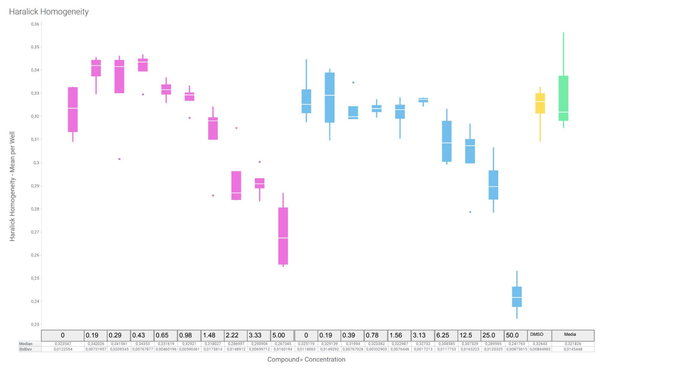

Fig. 4. Propidium Iodide intensity and Texture properties indicate compound and concentration dependent increase in cell death. (i) The PI intensity in the nucleus increases with compound concentration indicating cell death. In line with this, (ii) SER Texture property in the Calcein channel increases and (iii) Haralick Homogeneity decreases. Acetaminophen dose range = 0 - 5 mM in 1.5-fold dilutions, Troglitazone dose range = 0 - 50 µM in 2-fold dilutions

Result 4: Drug dose response of PH5CH8 and LX-2 cell cultures after 72 h treatment with Acetaminophen and Troglitazone indicates both drugs readily entered the encapsulated PH5CH8 and LX-2 coculture and markedly reduced cell viability.

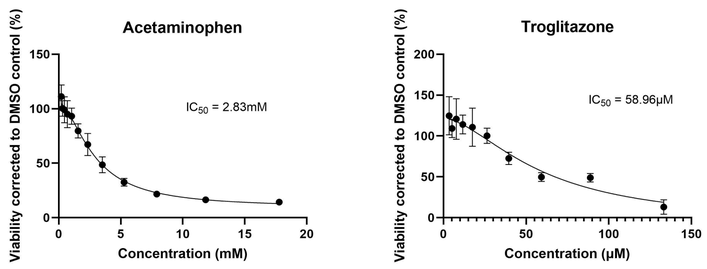

Fig. 5. In an independent experiment, IC50 were determined using CellTiter-Glo 3D cell viability assay (Promega). Data represented as mean+SD (n=4 technical replicates), 0.53% DMSO (as control) did not show any detrimental effect on cell viability.

Summary

We demonstrate that advanced cell models in a high-throughput 384-well format can be created simply, consistently and reproducibly, and are compatible with assays commonly used for drug treatment, and assessment of cell viability. This work shows how advanced cell models made with the RASTRUM platform can seamlessly integrate into existing workflows and downstream analyses for cytotoxicity studies, be scaled to high-throughput formats and be easily adapted to utilise primary cell types for more physiologically relevant cytotoxic responses. The combined use of RASTRUM platform and Revvity high content imaging and analysis systems can enable superior high throughput drug screening by which we can observe the effects of drugs in conditions closer to those found *in vivo* than is possible using conventional 2D or suspension culture systems.