Standardized, bioprocessed organoids enable automated platforms for high-throughput drug discovery.

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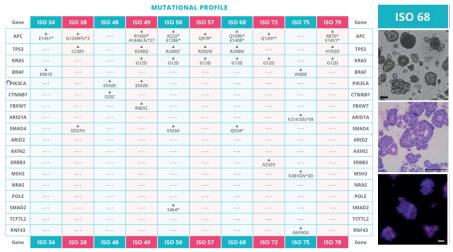
Introduction

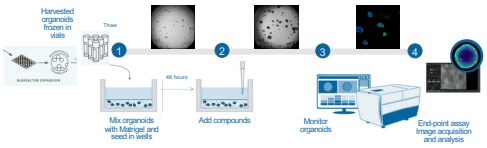
Patient derived organoids (PDOs or just organoids), represent a promising tool to reduce pipeline attrition in drug discovery. They fully represent the 3D architecture, cell-cell interactions and tissue microenvironment of the original tissue, including the cancer stem cell niches. Studies show that patients and their derived organoids respond similarly to drugs. PDOs are therefore an advanced and biologically relevant, in vitro model for the prediction of therapeutic efficacy and toxicity.

A semi-automated bioprocess has been developed by Cellesce, for the controlled production of standardized PDOs at scale, for use in high throughput screens.



Cellesce supplies vials containing 100,000 cryopreserved Colorectal Cancer (CRC) PDOs in an "assay ready format". Information such as mutational profiles, tumor characteristics and morphology are available for each line.

ISO68 CRC PDOs were seeded directly from the vials, into multi-well plates, manually or with an automated cell bioprinter and dispenser. They were treated with selected anti-cancer drugs and monitored over time using transmitted light imaging. For the analysis of size, texture, and additional morphological and phenotypic readouts, a deep learning-based image segmentation model was developed to facilitate automation of this process. A viability assay was carried out using live/dead cell dyes, and the PDOs were imaged in 3D on a high content confocal imager.



Workflow for using assay ready colorectal cancer organoids

Materials and Methods

Cell Culture

CRC PDOs (Cellesce) were handled according to the manufacturer's instructions. Briefly, the PDOs were thawed quickly at 37ºC, gently resuspended and washed in media. The organoid pellet was resuspended in Matrigel and seeded in a 384-well plate, at 200 organoids per well. Organoids were incubated with media containing ROCK inhibitor for 48 hours to improve recovery. Organoids were then treated with selected compounds for 5 days, at varying concentrations and in quadruplicate.

For automated seeding, the organoid Matrigel suspension was seeded into 96-well plates using the BAB400 (Advanced Solution) pipette tool. Gripper (PnP tool) Sequence can be used to de-lid and lid the plates, and to move the plates to the integrated IXM-C for imaging. 7µl of organoid suspension was dispensed in the middle of each well to form "domes". The automation path mapping the tool coordinates were set and recorded before the run.

High-throughput Imaging and analysis

The effects of compound treatment were monitored over time using the IXM-Confocal high content imager. CRC organoids were imaged using a 4X objective, with z-stacks enabled. For the viability assay, organoids were incubated with Hoechst, Calcein AM and ethidium homodimer for 2hrs at 37ºC. Images were acquired at 10X with Z-stacking. For staining with phalloidin, organoids were fixed in 4% PFA and then incubated with phalloidin 488. Images were then acquired at 10X or 20X (with water immersion objectives).

The INCarta image analysis software was used to analyze images acquired during monitoring. A deep learning-based approach was used to create a model for organoid segmentation.

Results

Assay set up with colorectal cancer organoids

To evaluate the use of the "assay ready" CRC organoids, a proof-of-concept study in a 384-well plate was designed. Organoids were thawed, mixed with Matrigel and seeded. After 48 hours, the CRC organoids were treated with a selection of nine compounds, at 7 concentrations and in quadruplicate.

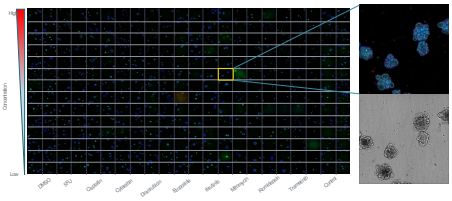


Figure 1. Assay setup. Plate map view of organoids in 384-well plate. Inset shows example image (top: fluorescent, bottom: transmitted light) from one of the wells. Organoids were labelled with Hoechst (nuclei, blue), phalloidin 488 (actin, green) and ethidium homodimer (dead cell marker, red).

Monitoring of phenotypic effects of compounds on organoids using deep learning

Unless genetically modified, patient derived cells and their organoids do not express fluorescent makers. Therefore, the ability to monitor cells and organoids in real time and extract meaningful information is dependent on robust image analysis of label-free transmitted light images. To monitor the quality of developing organoids, we used deep learning-based segmentation to analyze images acquired with brightfield imaging. Growth of CRC organoids can be monitored by measuring their diameters or areas over time. The effects of compounds on organoids can also be quantified. Here, the growth of CRC organoids was inhibited by romidepsin and trametinib.

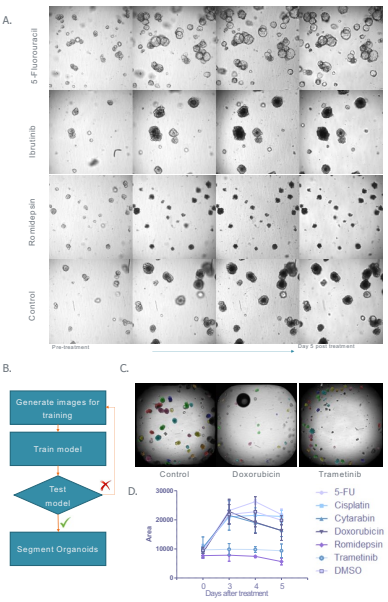


Figure 2. A) Example images of CRC organoids in Matrigel. Organoids were treated with the indicated compounds and monitored over 5 days. B) Overview of the SINAP workflow in IN Carta software to generate a model for organoid segmentation. C) Images acquired in transmitted light usually have high, non-homogenous background, edge effects and artifacts (such as bubbles) which prevents robust object segmentation. Shown here are example organoid images overlaid with a segmentation mask. A SINAP model was created to segment CRC organoids. D) Graph showing the change in average CRC organoid area over 5 days (error bars represent the standard deviation between replicate wells). Day 0 refers to images acquired pre-treatment.

Viability assay to quantify compound effects

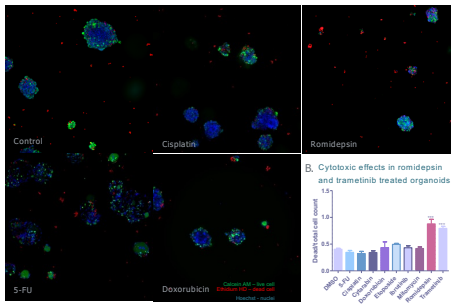


Figure 4: Effects of compounds on CRC organoids. A) Viability assay was carried out on CRC organoids after compound treatment. Organoids were stained with Calcein AM for live cells (green), ethidium homodimer for dead cells (red) and Hoechst for all nuclei (blue). Shown here are representative images of organoids. B) The ratio of dead cells (Hoechst and ethidium homodimer positive) for each compound shown. Romidepsin and trametinib treated organoids showed significant increase in dead cells compared to the controls ($p<0.001$)

Compound-induced phenotypic changes in CRC organoids

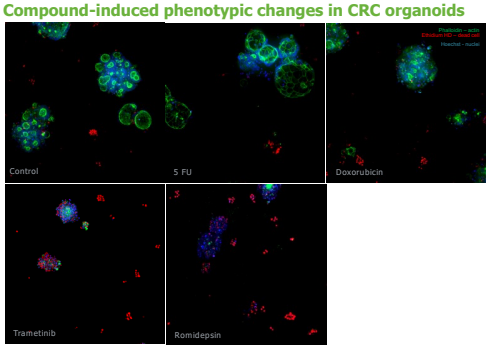


Figure 5. Phenotypic changes in CRC organoids. Organoids were fixed and stained with phalloidin after 5 days following compound treatment.

Automation of CRC organoids seeding

Automation of the CRC cell seeding was carried out using the BAB400 bioprinter. CRC organoids were mixed with Matrigel and then seeded in a dome in the middle of the each well in a 96-well plate.

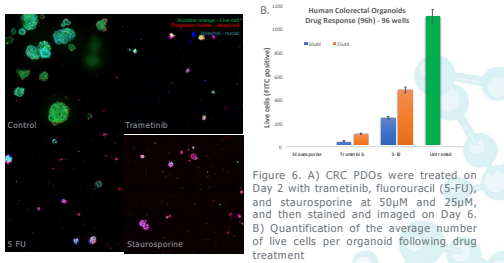


Figure 6. A) CRC PDOs were treated on Day 2 with trametinib, fluorouracil (5-FU), and staurosporine at 50µM and 25µM, and then stained and imaged on Day 6. B) Quantification of the average number of live cells per organoid following drug treatment

Conclusion

- Our results support the use of PDOs for high throughput assays such as compound screening. The availability of standardized, assay ready PDOs provides significant time savings for screens using 3D models
- We show that an AI-based approach can be successfully be used to generate robust segmentation for the analysis of label-free biological modes such as 3D organoids.