Development of a 3D high-content imaging based explorative toxicology platform for tumor and healthy colorectal patient-derived organoids

Peter van Schaik[1], Cinthya Del Angel Zuvirie[1], Femke Huijer[1], Lois van der Drift[1]. Hester Bange[1], Marten Hornsveld[1], Maria Garcia-Collado[2], Maryia Karpiyevich[2]

[1]Crown Bioscience Netherlands BV, Leiden, the Netherlands, [2]SPT Labtech, Cambridge, UK

Abstract

Explorative in vitro toxicology models are important for improving early safety assessment, while traditional systems may lack physiological relevance. Patient-derived organoids (PDOs) represent a significant advancement because they mimic key clinical relevant aspects of epithelial tissue biology in a 3D environment.

This study describes the development of an explorative toxicity screening platform using colorectal PDOs obtained from both tumor and matched healthy tissues. The latter is organized into distinct stem cell and differentiated cell compartments with differing proliferative and functional roles, where stem cells maintain homeostasis and self-renewal, and differentiated cells perform specialized functions including drug uptake and barrier maintenance.

A high-throughput assay was established to assess compound-induced toxicity in GI organoids enriched for either stem cell or differentiated cell populations, alongside corresponding tumor PDOs. Using the SPT Labtech firefly® platform, an automated workflow was developed to perform organoid seeding, medium handling and compound treatments. Using the firefly's positive displacement dispensing heads, PDOs were seeded from cryopreservation in a 3D hydrogel and supplemented with model-specific organoid culture medium. After a 48h recovery period, a medium exchange was performed to replace the organoid culture medium with differentiation medium. Additional medium refreshes were performed 120h post seeding. A panel of 6 compounds; Afatinib, Adagrasib, DM4, DXD, Gemcitabine and Bosutinib, was used to assess toxicity in 11 PDOs and to identify changes in response compared to differentiated organoid models. The effects of compounds were visualized using high content imaging of the nuclei and F-actin cytoskeleton. Image analysis (IA) was performed to measure the change in phenotypic characteristics, including organoid and nucleus size and count, cells per organoid and organoid shape, to enable distinction between cytotoxic and cytostatic compound-induced effects.

IA identified morphological differences in PDOs between differentiated and undifferentiated states. In healthy colorectal models, differentiation was associated with a reduction in lumen size and an increase in actin cytoskeletal complexity. In contrast, colorectal tumor derived models exhibited more heterogeneous morphological alterations, likely reflecting underlying intrinsic tumor biology.

The combination of automations in liquid handling, tissue differentiation and image processing offers a framework for establishing the therapeutic window of oncology drugs associated with diarrhea and GI toxicity, that uniquely facilitates the independent evaluation of compound effects on the healthy stem cell and differentiated compartments, as well as on tumor tissues.

Future work will focus on expanding the range of indications with available Breast, Lung and Pancreatic tumor and healthy organoid pairs and increase assay throughput. This platform provides a foundation for evaluating modern therapeutics such as antibody-drug conjugates (ADC's), PROTACs and small molecules with known high toxicity profiles.

Introduction

Explorative in vitro toxicology models are important for improving early safety assessment, while traditional systems may lack physiological relevance. Patient-derived organoids (PDOs) represent a significant advancement because they mimic key clinical relevant aspects of epithelial tissue biology in a 3D environment. This study describes the development of an explorative toxicity screening platform using colorectal PDOs obtained from both tumor and matched healthy tissues. A high-throughput assay was established to assess compound-induced toxicity in Gastrointestinal (GI) organoids enriched for either stem cell or differentiated cell populations, alongside corresponding tumor PDOs. Using the SPT Labtech firefly platform, an automated workflow was developed to perform organoid seeding, medium handling and compound treatments.

Methods

- Eleven PDO models, including five colorectal tumor (ending in '-B'), four colorectal healthy (ending in '-D'), and two healthy ileum models, were seeded in a 3D hydrogel matrix using the SPT Labtech firefly platform.
- Each model was cultured under two media conditions: standard culture medium and differentiation medium. PDOs were maintained in either condition for 120 hours with regular media refreshes to support growth and differentiation.
- Following 72 hours of compound exposure, organoids were fixed with paraformaldehyde (PFA) and stained for nuclei (Hoechst) and F-actin (Rhodamine Phalloidin). High-content imaging Z-stack acquisition was performed to quantify treatment-induced phenotypic and morphological effects.

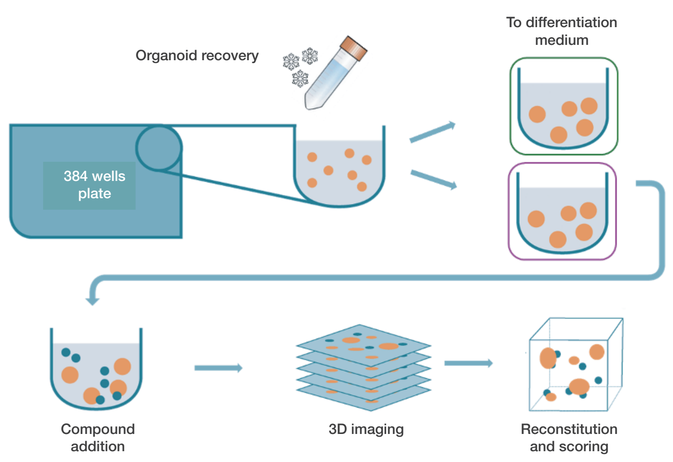

Figure 1. Procedure

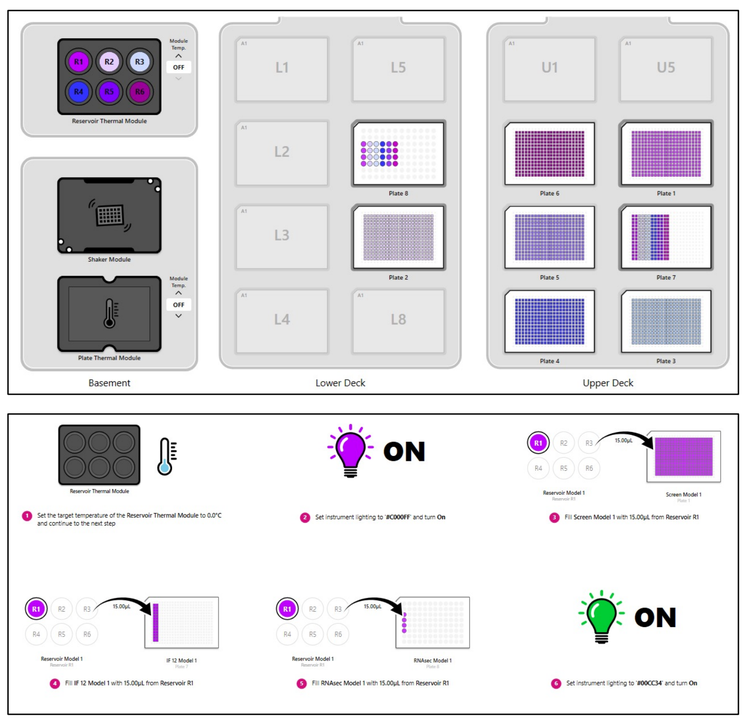

Figure 2. firefly deck layout & details

Results

To ensure accurate dispensing and robust assay performance, the accuracy and precision of a range of dispense volumes were evaluated using the positive displacement syringe. Using standard dispensing settings, accuracy values below 3% were achieved for volumes of 4 µL and higher, while precision values below 3% were obtained across a dispensing range of 200 nL to 40 µL, including viscous liquids. These results demonstrate reliable and reproducible dispensing performance independent of volume or liquid type.

Volume	Accuracy	Precision (%CV)
40 µL	-0.59	1.34
4 µL	2.99	1.16
0.2 µL	n/a	2.73
15 µL (viscous liquid)	n/a	1.22

Accuracy was assessed gravimetrically and expressed as the percentage deviation from the target dispense volume. Precision was evaluated using Orange G diluted in PBS, with absorbance measurements acquired using a plate reader. All measurements were generated from 384 dispenses (one full plate) for each validated volume using a single syringe configuration.

To assess the reliability of organoid seeding in hydrogel, a test seeding experiment was performed to evaluate intra-plate and inter-well variability. Using the average TRITC intensity of Rhodamine Phalloidin–stained F-actin as the quantitative readout, a coefficient of variation (CV) of 2.46% was observed across 240 seeded wells. Top- and side-view image projections further demonstrated uniform distribution of both hydrogel and organoids throughout each well, indicating excellent seeding quality in 384-well plates.

This uniform distribution enables reliable segmentation and downstream image analysis, while organoids maintained structural integrity following dispensing. Together, these results demonstrate the suitability of the workflow for robust high-content imaging and reproducible dose-response analysis.

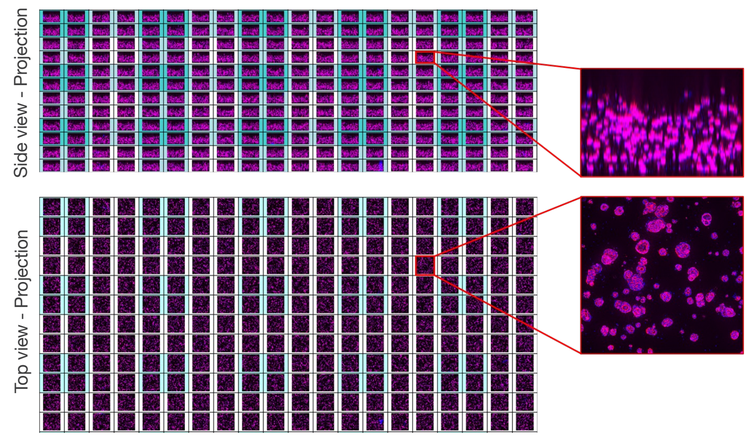

Figure 3. Organoid Seeding consistency

Finally, the assay quality was assessed by calculating the Z' of each model, in different growth conditions, using the cytotoxic and solvent controls. Consistent Z' values of >0.55, using the fraction of dead cells as measurement, indicated good assay performance and reliable dispensing by the firefly.

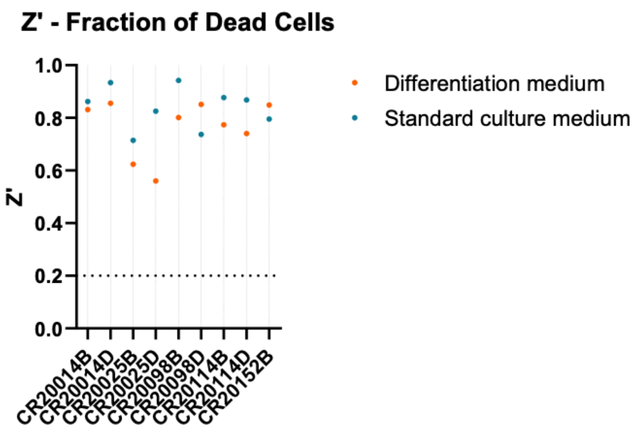

Figure 4. Assay Quality

Successful differentiation of PDO models was achieved using differentiation medium, as illustrated in Figure 5. In healthy models CR20014D, CR20025D, and CR20098D, lumen size was used as a marker of epithelial differentiation and was found to be reduced in organoids cultured in differentiation medium compared to those maintained in standard culture medium. This differentiation-associated effect was not observed in tumor models CR20014B and CR20098B. In contrast, tumor model CR20025B displayed an increase in lumen size following culture in differentiation medium.

Additionally, total F-actin intensity, used as an indicator of cytoskeletal maturation, was increased in healthy models CR20014D, CR20025D, and CR20098D grown under differentiation conditions relative to standard culture conditions. Conversely, matched tumor models showed a decrease in total actin signal upon differentiation treatment.

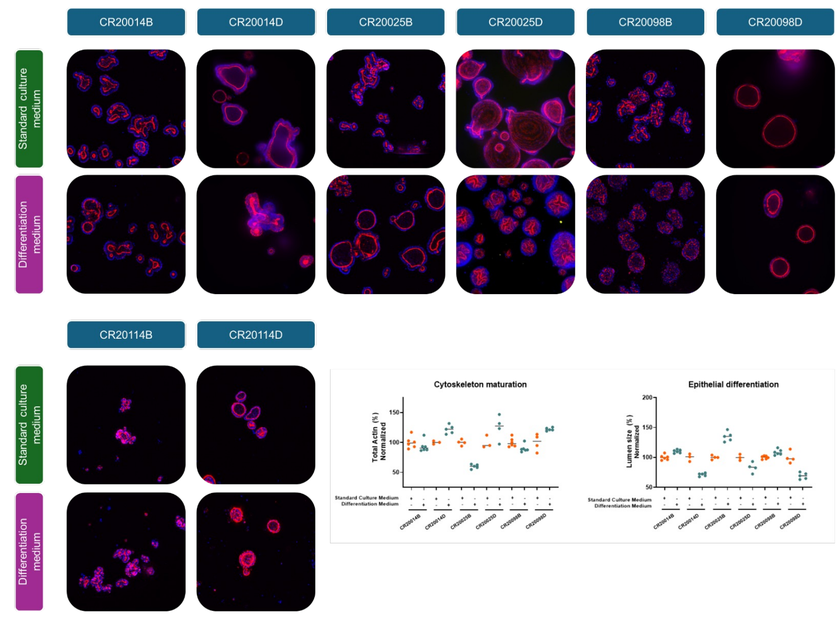

Figure 5. Differentiation

Conclusion

In conclusion, this study demonstrates the successful development of a robust high-content imaging assay using matched tumor and healthy colorectal PDOs for toxicity screening. Automated positive displacement dispensing enabled reproducible organoid seeding with low variability across 384-well plates. Furthermore, differentiation-induced phenotypic changes were successfully detected in healthy PDOs, highlighting the biological relevance of the platform. Together, these results establish a scalable and physiologically relevant workflow suitable for evaluating modern therapeutic modalities and assessing therapeutic window in complex 3D models. Future work will focus on expanding the range of indications with available Breast, Lung and Pancreatic tumor and healthy organoid pairs and increase assay throughput. This platform provides a foundation for evaluating modern therapeutics such as antibody-drug conjugates (ADC's), PROTACs and small molecules with known high toxicity profiles.