Breaking Barriers: Expanding Drug Toxicity Modeling through Automated Intestinal Organoids for Enhanced Diversity Representation in the Human Intestinal Epithelium

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ABSTRACT

Predicting clinical gastrointestinal (GI) toxicity during preclinical drug development has long been a significant challenge. Traditional toxicology models, relying on laboratory animals or transformed monoculture cell lines, often fall short in accurately recapitulating human physiology and predicting the diverse range of clinical responses. In recent years, tissue-derived intestinal epithelial organoids have emerged as a physiologically relevant in vitro model that retains the genetic and epigenetic variability observed in donors. However, the widespread adoption of organoids has been hindered by challenges such as manual manipulation, variability in culture conditions, and scalability for high-throughput screening.

In this project, we present an innovative approach to overcome these challenges by developing an automated arrayed culture system for patient-derived organoids. Leveraging state-of-the-art liquid handlers, we address these hurdles and enhance the efficiency, robustness, and flexibility of our organoid culture system. By implementing arrayed cultures, utilizing standard labware, and optimizing harvest and digestion techniques, we have overcome manual culture limitations and facilitated the scaling up of multiple organoid lines. This innovative approach enables organoid-based high-throughput screening that we will apply to this study to conduct a comprehensive high-throughput toxicology screen in human intestinal organoids. By doing so, we aim to establish a robust framework for inclusive preclinical toxicity testing, laying the groundwork for future diversity studies that hold the potential to significantly enhance our understanding of drug efficacy, disease biomarkers, and pharmacodynamics.

INTRODUCTION

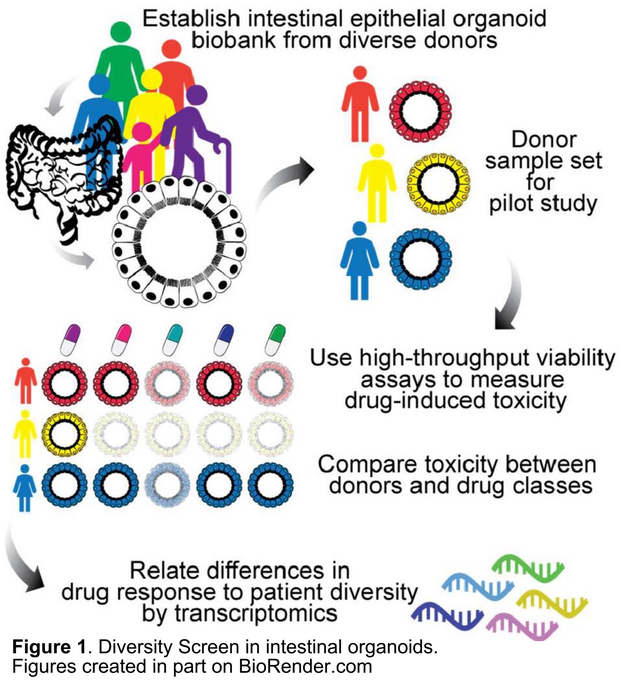


Figure 1. Diversity Screen in intestinal organoids. Figures created in part on BioRender.com

Gastrointestinal toxicities (GIT) in Phase 1 clinical trials burden patients, requiring additional treatments and dose reductions. However, accurately representing the human gastrointestinal system in preclinical models is challenging. Traditional approaches like rodent studies and monolayer cultures have limitations in capturing gut complexities.

Tissue-derived intestinal epithelial organoids offer a promising in vitro model, preserving genetic and epigenetic variability. However, the lack of diversity in donor cell lines limits generalizability to the global population. To address this, integrating donor diversity is crucial, considering the impact of genetic and epigenetic variations on drug responses and disease outcomes. Current organoid models have variability in culture conditions, manual manipulation, and integration into high-throughput methods. To overcome these limitations, we present an automation solution using liquid handlers. This solution addresses miniaturization, 3D culture design, and cellular heterogeneity, enabling organoid-based high-throughput screening.

TOXICITY SCREEN DESIGN

3D Intestinal Organoid lines exibit cellular diversity of the original tissue[5]. The culture of each donor is used to seed 2D Transwell plates, which are amenable to multiple readouts: 1) cell viability and 2) Barrier function [3]

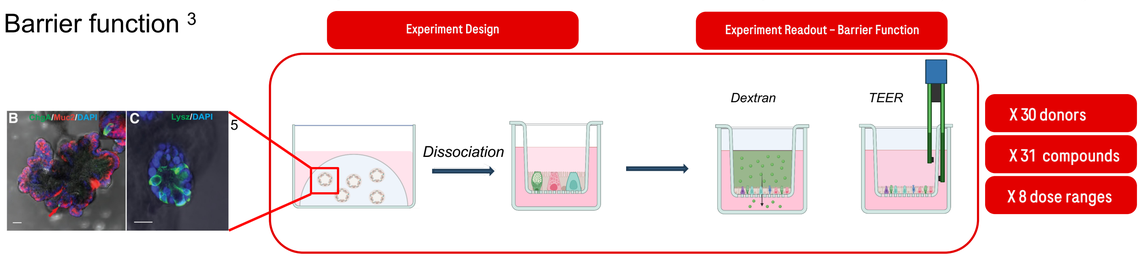


Figure 2. Diversity Screen workflow in intestinal organoids. Organoids eFigures created in part on BioRender.com

SYSTEM & METHODS

For our organoid culture development, we utilize an integrated Tecan Fluent liquid handler, which is equipped with an array of third party devices that are all controlled via the Fluent software.

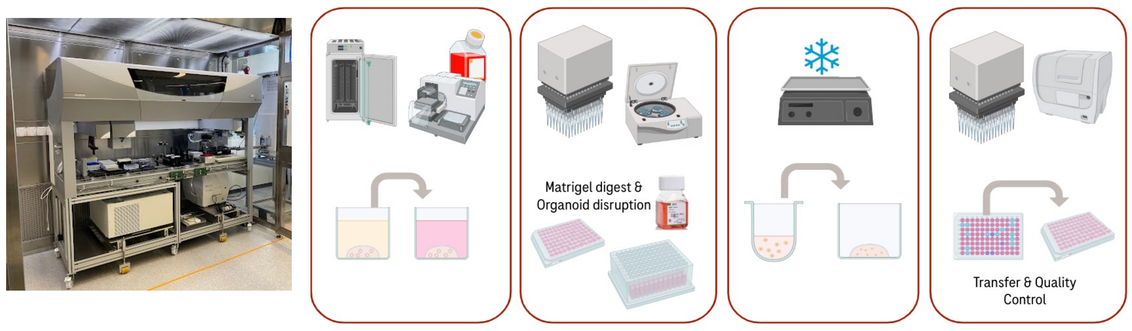


Figure3. Integrated Tecan Device:
Third party devices and their use in the different steps of the workflow: Bsl2 Enclosure, Heater Shaker, Chilled Centrifuge, Automated Microscope, Plate Washer, Automated Incubator. Figures created in part on BioRender.com

The Fluent is equipped with a 96 EVA or 384 Multi-Probe Head, 8 air-based liquid handling channels, and a robotic arm for labware movement.

To cultivate intestinal organoids, we employ hydrogel domes, with matrigel being our hydrogel of choice. Matrigel remains in a liquid state below 6°C and solidifies at higher temperatures. These domes are then seeded onto flat bottom plates.

To ensure flexibility and adaptability, we divide our workflow into the following steps:

1. Cell seeding of matrigel domes in 96-well flat bottom plates.
2. Maintenance media exchange in 96-well flat bottom plates.
3. Cell splitting: Harvest and organoid disruption.
4. Assay treatment and quality control.

The scripts are programmed to address either full 96-well plates or user-defined columns of the plate.

AUTOMATION WORKFLOW

To ensure system robustness, we developed a Matrigel-based organoid culture that is robust, flexible, and modular, allowing for easy adoption on different technology platforms (e.g., Hamilton, Opentron). This facilitates technology transfer and wider application of the workflows. To achieve this, we took the following crucial steps:

1) Avoiding specialised labware:
We switched to standard tissue culture flat bottom 96-well plates instead of non-standard ones. We also found alternative solutions to wide bore tips to avoid dependence on a specific producer.

2) Harvest and Digest Optimization:
We optimized the Matrigel dissociation process for efficiency, gentle cell treatment (enzyme-free), and room temperature compatibility. Manual harvesting of Matrigel domes can be challenging to transfer to an automation system, so we found that Corning Recovery Solution works best agent for releasing Matrigel using mixing cycles near the bottom of the well and at room temperature.

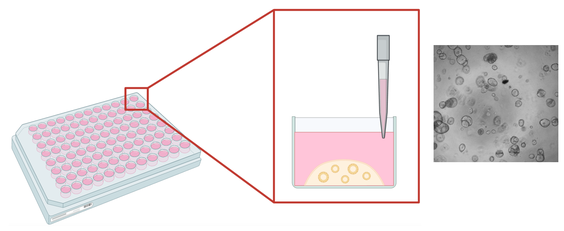
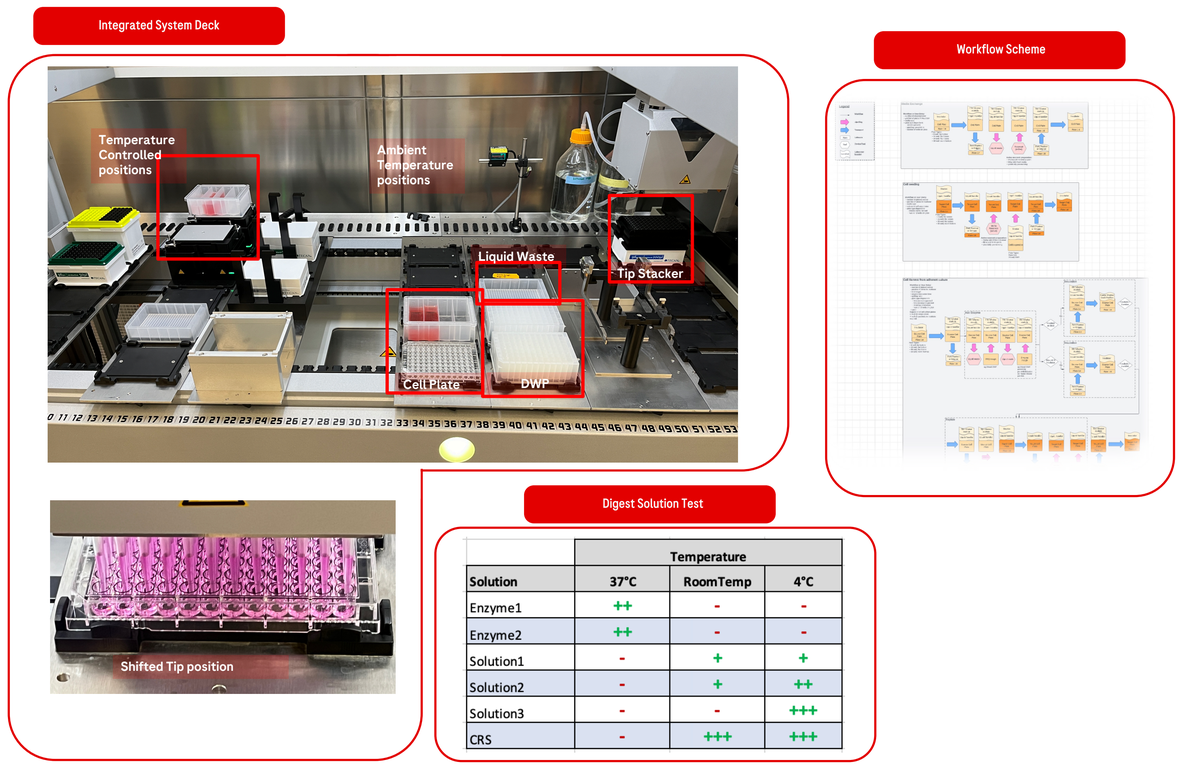


Figure 4. Scheme of Matrigel dome culture in 96 flat bottom plate and pipetting strategies

3) Scale Up: With our integrated device, we can upscale and parallelize multiple organoid lines.

These steps ensure the robustness of the system, facilitate adoption on different platforms, optimize the Matrigel dissociation process, and enable scaling up of organoid culture.



Solution	Temperature 37°C	RoomTemp	4°C
Enzyme1	++	-	-
Enzyme2	++	-	-
Solution1	-	+	+
Solution2	-	+	++
Solution3	-	-	+++
CRS	-	+++	+++

Figure 5. Details of Integrated Tecan Deck and Workflow 96 MPH processing a culture plate, Dissociation reagent comparison, scheme of workflow of Tecan scripts, strategy for upscaling patient samples

CONCLUSIONS

- **We established an automated workflow that allows for parallel- arrayed organoid culture of diverse patient lines.**
- **Our Workflow will enable organoid-based high-throughput screening in diverse patient derived samples**

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