Bespoke QC assay using real-time quantitative imaging for intestinal organoids biobank

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INTRODUCTION

The human intestinal epithelium consists of numerous cell types that all contribute to the healthy functioning of the human gut. New epithelial cells are produced by stem cells located in crypts at the base of the intestinal glands. These stem cells give rise to progenitors that differentiate into mature epithelial cell types as they migrate up the crypt-villus axis.

In healthy individuals, the intact mucus layer prevent bacterial invasion of the epithelial layer. However, in patients with Inflammatory Bowel Disease (IBD), the mucosal layer is damaged, leading to a dysfunctional barrier. This allows for bacterial penetration, which induces the imbalance of immune regulation that leads to a chronic inflammatory state.

We aim to create a large biobank of organoids from IBD patients to interrogate the underlying causes of IBD and create more efficient drugs. The created organoid lines require rigorous QC process to evaluate their propagation and differentiation potential.

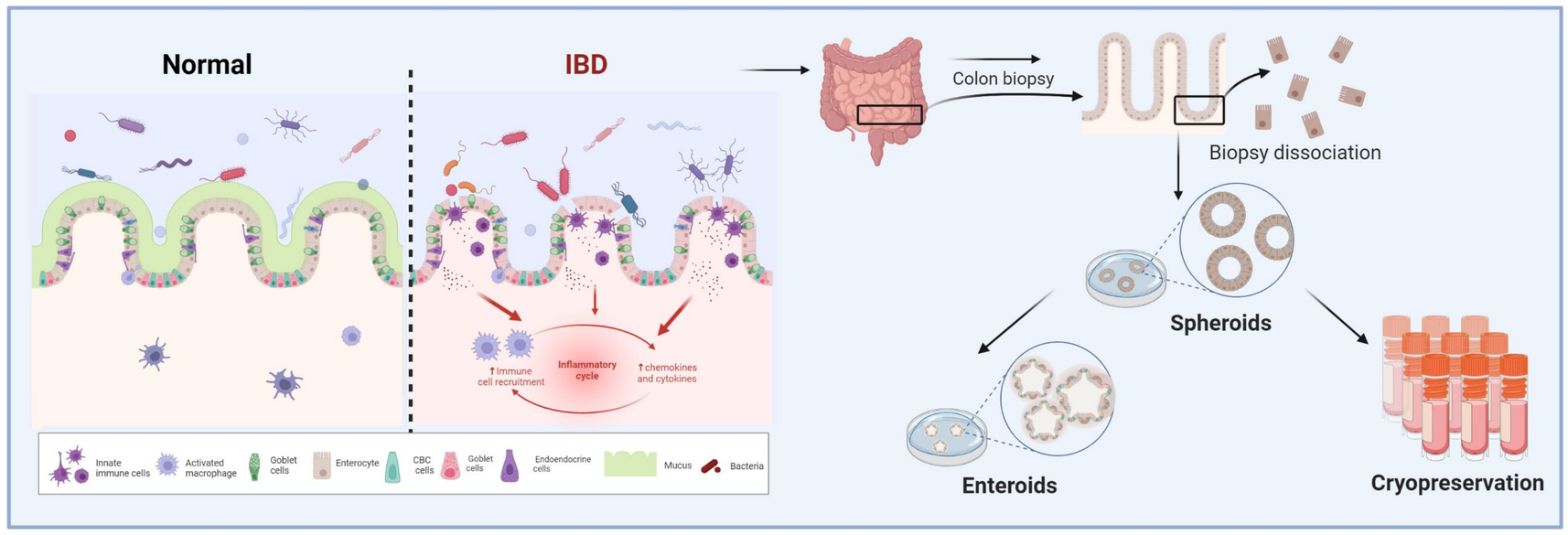


Figure 1 Intestinal epithelial stem cells from biopsies of healthy individuals and IBD patients that are dissociated and plated into ECM matrix have the ability to self-organise to form spheroids. Spheroids can be differentiated into enteroids ('mini-guts') that are used as models of the intestinal epithelium for functional validation studies. Large quantities of spheroids are cryopreserved to create a biobank, which will be used for future screening and functional studies. The cryopreserved samples need to undergo rigorous QC process to ensure the quality of the resource.

METHOD

The main criteria of organoid lines to be validated by QC:

1. Viability upon thawing
2. Aseptic culture
3. **Propagation potential**
4. **Differentiation potential**

Key risk: Not enough stem cells in the organoids derived, leading to compromised propagation potential.

How many QC passages in culture do you need to determine if there is a problem with the propagation of an organoid line?

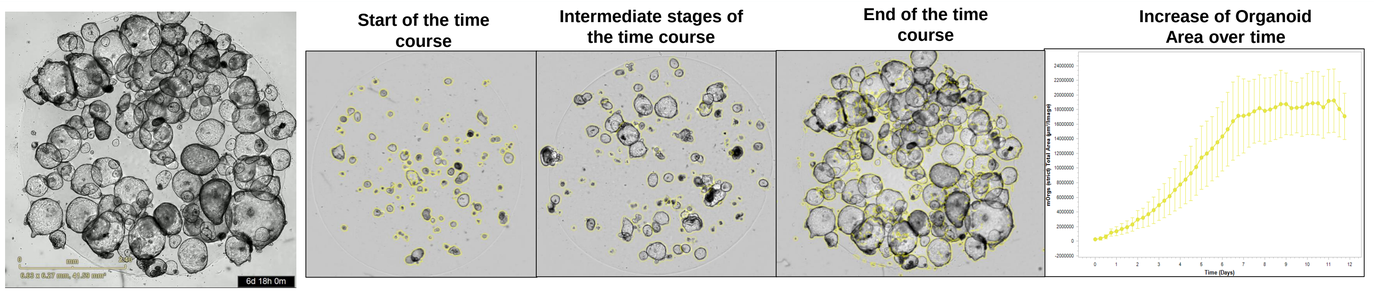


Figure 2 Live imaging of the intestinal spheroid growth on Incucyte SX5 allows automated label-free analysis to quantify the kinetics of the organoid expansion over time. HD max projection images of whole organoid ECM domes are subjected to rigorous quantification protocol that measures the increase in the organoid area over time.

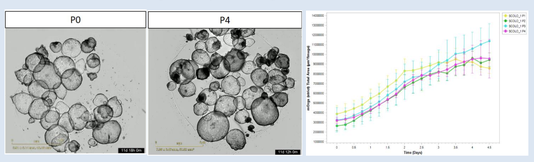
RESULTS

*The morphology and stem cell content of primary tissue-derived intestinal organoids at the stage of cryopreservation varies greatly. Visually, the organoid cultures can be identified as having **primarily cystic morphology (stem cell-rich lines), primarily budding morphology (stem cell-poor lines) and mixed** morphology. Using real-time automated live imaging we found that organoids in different morphological states have distinct propagation kinetics that affect their differentiation potential.*

STEM-CELL RICH LINE

PROPAGATION ASSAY

Comparing proliferation kinetics of a cell line at a range of passages after thaw

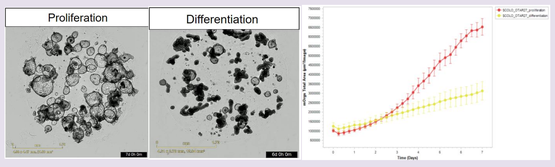


One-Way ANOVA *p*-value is .85. The result is *not* significant at $p < .05$.

The propagation rate of an intestinal stem cell rich line stays the same regardless the number of passages post-thaw

DIFFERENTIATION ASSAY

Comparing area growth of a cell line in propagation and differentiation media after thaw:



One-Way ANOVA p-value is .00499. The result is significant at $p < .05$.

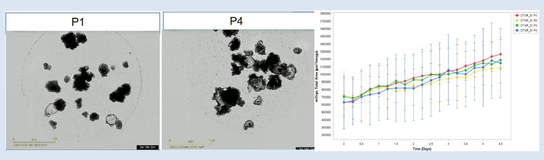
The differentiation potential of an intestinal stem cell rich line is robust immediately post-thaw but becomes more pronounced with more time in culture

The intestinal organoid lines that are **stem cell rich** can show evidence of effective propagation and differentiation without needing much time in culture (P0 or P1 after thaw)

STEM-CELL POOR LINE

PROPAGATION ASSAY

Comparing proliferation kinetics of a cell line at a range of passages after thaw

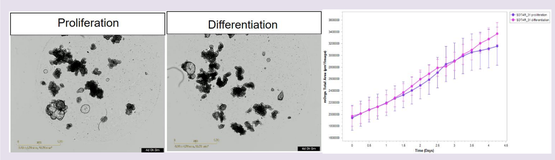


One-Way ANOVA *p*-value is .371195. The result is *not* significant at $p < .05$.

The propagation rate of an intestinal stem cell poor line does not improve despite a number of passages in culture post-thaw

DIFFERENTIATION ASSAY

Comparing area growth of a cell line in propagation and differentiation media at P4 after thaw:



One-Way ANOVA *p*-value is .723485. The result is *not* significant at $p < .05$.

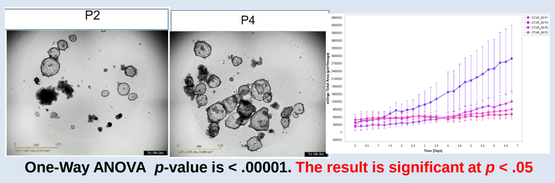
The differentiation potential of an intestinal stem cell poor line is deficient, as only a small percentage of stem cells is present in the organoids

The intestinal organoid lines that are **stem cell poor** might not be able to demonstrate effective propagation kinetics, but could be maintained in culture using low passaging ratios

MIXED MORPHOLOGY LINE

PROPAGATION ASSAY

Comparing proliferation kinetics of a cell line at a range of passages after thaw

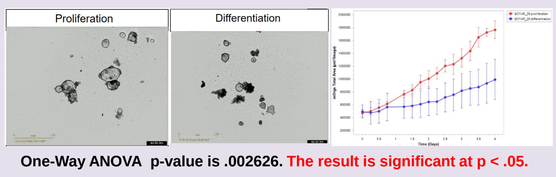


One-Way ANOVA *p*-value is < .00001. The result is significant at $p < .05$

The propagation rate of a mixed morphology line improves after several passages post-thaw. Proliferation media selects for the presence and expansion of the stem cell population

DIFFERENTIATION ASSAY

Comparing area growth of a cell line in propagation and differentiation media at P4 after thaw:



One-Way ANOVA p-value is .002626. The result is significant at $p < .05$.

The differentiation potential of a mixed morphology line is improved once the intestinal stem cell population is enriched in the organoids.

The intestinal organoid lines that contain organoids **at various levels of 'stemness'** might not be able to demonstrate effective propagation kinetics right after thaw, but will build up the presence of intestinal stem cells over several QC passages. The resulting population ends up showing excellent propagation and differentiation kinetics

CONCLUSION

The implementation of QC grading for organoid lines:

Grade A:

- The line has **mostly cystic morphology after thaw**
- The Incucyte proliferation and differentiation assay can be **set up at P1 after thaw**.
- The kinetics of proliferation/ differentiation are **in line with our expectations and statistically significant**

Grade B:

- The line **needs several QC passages until it reaches mostly cystic morphology**
- The Incucyte proliferation and differentiation assay **set up at or before P4 after thaw**.
- The kinetics of proliferation/ differentiation are in line with our expectations and statistically significant

Grade C:

- The line **doesn't reach mostly cystic morphology up to P4**
- The Incucyte kinetics of proliferation/ differentiation are **NOT in line with our expectations and NOT statistically significant**
- The line can be maintained in culture for 4 passages using low passaging ratios

Grade D: FAIL

- The line **fails thawing process**
- The line is **contaminated**
- The line **cannot be maintained in culture** for four passages using low passaging ratios