Development of Mucosal Organoid Biobank as a Disease Model Platform for IBD Research

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Project Overview

Organoids are 3D cellular models consisting of self-organised stem cells. This project produces organoid models derived from intestinal stem cells (ISCs). ISCs are located in the crypts of the villi lining of the intestines. These stem cells are extracted and cultured to grow *in vitro* to form organoids. The cells are derived in Intesticult media with ROCK Inhibitor. The media is selective for the ISCs and the ROCK Inhibitor increases their survivability.

The current aim of the project named **"mOrganoids"**, is to create a biobank of 400 intestinal organoid models, each containing at least 1.5 million viable cells. The biobank will consist of organoid lines derived from surgically extracted biopsies from both healthy patients and patients with Inflammatory Bowel Disease (IBD)such as Crohn's Disease and Colitis. Since intestinal organoid models partially recapitulate the identity, cell heterogeneity and cell behaviour of the original tissue *in vitro*, the biobank will address many fundamental questions regarding the pathophysiology of the disease, such as low production of **secretory (mucosal) progenitors** in IBD patients.

Furthermore, this Biobank will provide the opportunity to investigate genetic variants impact on the cellular response, gene expression and identification of IBD cellular phenotypes. Therefore, creating a large intestinal organoid biobank provides a necessary and personalised model to expand the understanding of IBD.

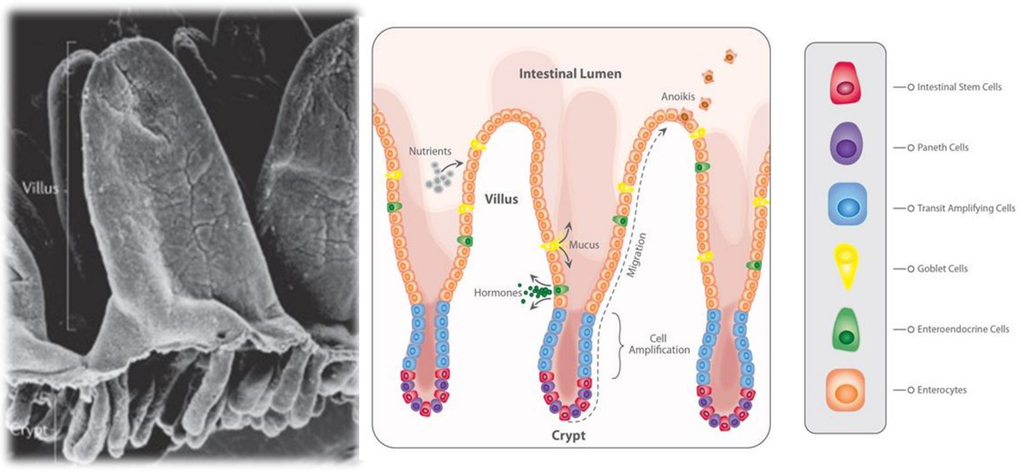

Figure 1. The small intestine epithelial and its respective cell types.
1) (left) Barker, N. (2013). Adult intestinal stem cells: Critical drivers of epithelial homeostasis and regeneration. *Nature Reviews Molecular Cell Biology*, *15*(1), 19–33. https://doi.org/10.1038/nrm3721. **2)** (right) *Intestinal Organoid Mini-Review*. STEMCELL Technologies. (n.d.). Retrieved December 6, 2022 from https://www.stemcell.com/technical-resources/area-of-interest/organoid-research/intestinal-research/mini-review.html

mOrganoids Pipeline

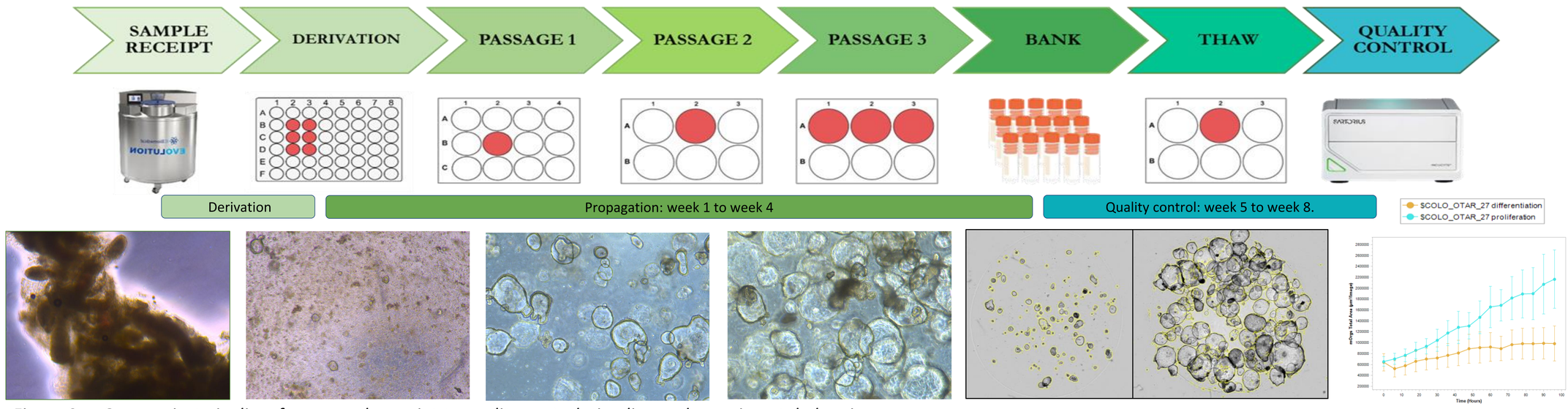

Figure 2. mOrgs project pipeline, from sample receipt to quality control: timeline and experimental planning.

The **project pipeline** (***Fig.2***) extends over a time period of 2 months. Before arrival of the biopsies the donor blood is genotyped to gather the background genetic information of the sample. The intestinal biopsies collected from the *Anderson faculty* and placed in LN2 tanks for storage. Within 3 months of collection, biopsies undergo derivation, a process through which the new donor-specific organoid cell lines are generated. During derivation the biopsies are subjected to physical and chemical stresses in order to extract the live ISCs that are subsequently plated in 3D matrigel scaffolds to allow organoids formation. Prior to expansion, the organoids are tested for mycoplasma. The derived organoids will then be expanded through passages, in increasing culture volumes for the following month, to establish a high-quality viable biobank .

Due to the inherent differences between the received biological samples, mucosal organoids can have **two distinct morphologies** during (***Fig.3***). In some lines the abundance of proliferating ISCs allows for the formation of cystic organoids while in others the presence of differentiating stem cells gives rise to gut-like formations less prone to expansion. At the end of the propagation phase, the organoids in culture are assayed for cell number and viability and then banked.

Finally, the organoids undergo **Quality Control Assessment**. The QC process involves growing lines for a further number of passages to ensure that they are viable upon thawing, contamination free and able to propagate and differentiate. The organoids that demonstrate standard growth rates and differentiation patterns with the Incucyte assay are then graded on an A to D scale, with A being assigned to lines that are mostly cystic and stem cell rich and D to the lines that fail to model intestinal organoids growth.

The use of **fresh biopsy samples** in this pipeline is currently being validated to improve the success rate of organoid derivation from disease sample.

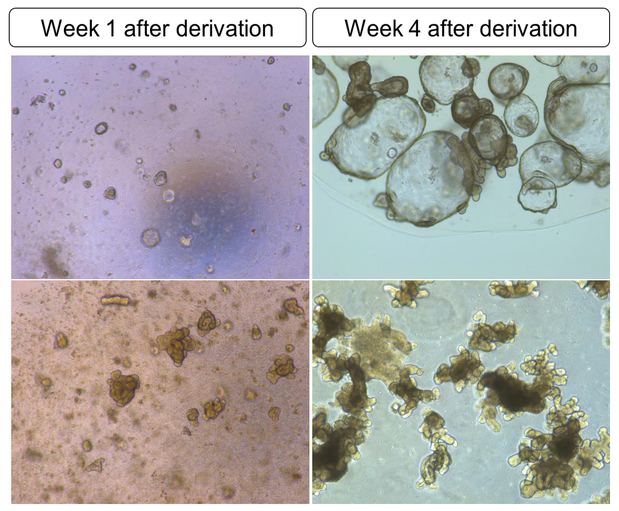

Figure 3. Different morphologies in mucosal organoids: cystic vs differentiating.

Current achievements

Category	Total Numbers (since March 22)
Processed	287
Banked	180
Failed	97
Average Success Rate	66.2%
QC	21
In the primary pipeline	10

Objectives Timeline

The aim is to **bank 400 mucosal organoids over the 3 years**, with an average flow rate of 2-3 biopsies derived per week. Allowing for a 37% failure rate, more than 600 biopsies will be derived over the project timeline. As the pipeline becomes more refined, the capability of increasing the number of derivations per week becomes achievable.

Acknowledgements

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