A Serum-free Medium For Expanding Patient-derived Cystic Fibrosis Rectal Organoids And Performing Functional Assays

Roxana Mustata-Micsik[1]*, Johanna Pott[2]*, Marianne Lankhorst[3], Nilofar Ehsani[2], Javier Frias-Aldeguer[2], Sharon A. Louis[3], Allen C. Eaves[3,4], Sylvia F. Boj[2], and Ryan K. Conder[3]

[1]STEMCELL Technologies UK Ltd., Cambridge, UK; [2]Hubrecht Organoid Technology, Utrecht, the Netherlands; [3]STEMCELL Technologies Inc., Vancouver, BC, Canada; [4]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; * Equal contribution

INTRODUCTION

Current media formulations for human intestinal patient-derived organoids (PDOs) rely on the use of serum-containing conditioned media (CM) to ensure Wnt activity. The undefined components in serum and CM can introduce performance variability in supporting organoid growth and functional assays. The quality control and batch variability of the CM, as well as cell line contamination risk, constitute a manufacturing burden for labs across the world. Additionally, the serum proteins in culture medium can bind to drugs and affect the drug activity during functional assays. To address this we used IntestiCult™-SF Organoid Growth Medium (IntestiCult-SF OGM), a serum-free and conditioned medium-free medium to expand biobanks of rectal PDOs with mutations in the CFTR gene (CF) and to validate IntestiCult-SF OGM for Forskolin-induced swelling assay in CF PDOs.

METHODS

Patient derived organoids from normal donors or donors with different CFTR genotypes were thawed from cryostocks and expanded over 3 passages in Wnt-CM based medium or in IntestiCult™-SF OGM. Each organoid culture was thawed from a single vial and expanded separately for every medium condition for at least two passages before the start of the experiments. One CF PDO and one normal PDO were harvested for histological assessment and qPCR analysis. Forskolin-induced swelling (FIS) assay was performed for three CF cultures in Wnt-CM based medium and IntestiCult-SF OGM culture medium as follows: cultures were seeded in parallel in a 96-well plate and incubated with VX-661 (3 µM, 24 hours before imaging), VX-445 (0.3 µM, 24 hours before imaging) and VX-770 (3 µM, dispensed with Forskolin). Organoid swelling was measured over 60 minutes after the addition of VX-770 and Forskolin (DMSO control, 0.02 µM, 0.05 µM, 0.128 µM, 0.32 µM and 0.8 µM). For long term culture maintenance experiments, four CF PDOs were thawed and expanded in IntestiCult-SF OGM for 2 passages, then freeze in liquid nitrogen, shipped on dry ice, and thawed in IntestiCult-SF OGM medium. CF organoids were weekly passaged in IntestiCult-SF OGM for at least 10 weeks.

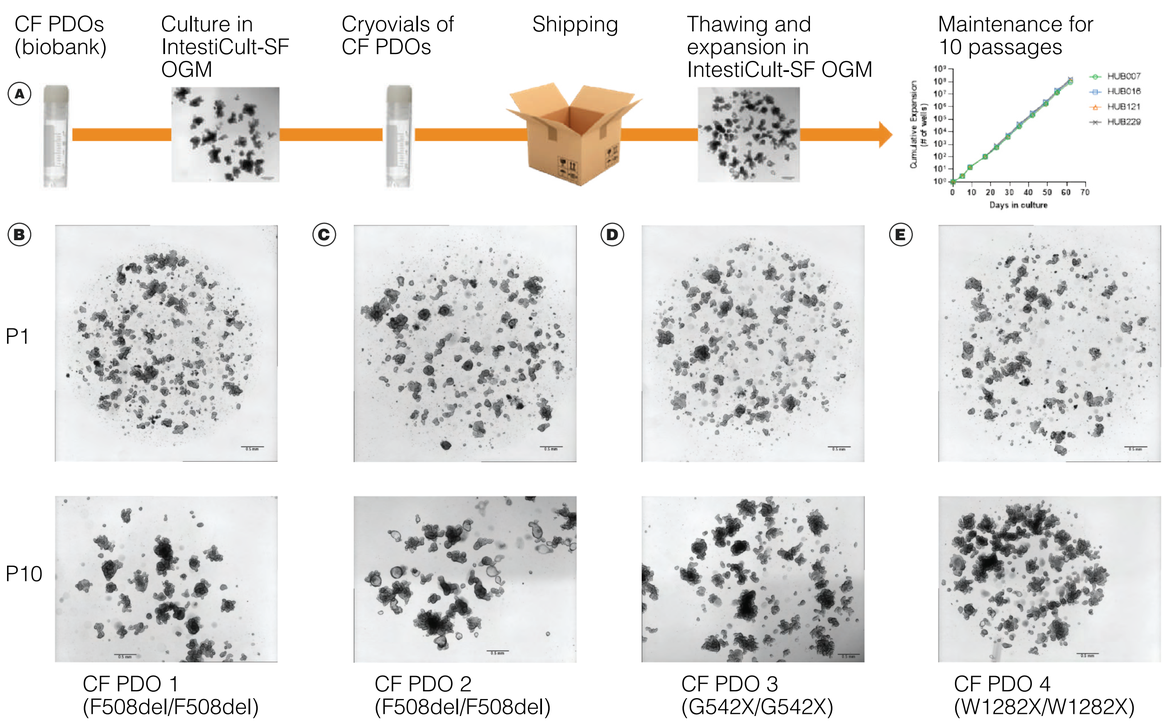
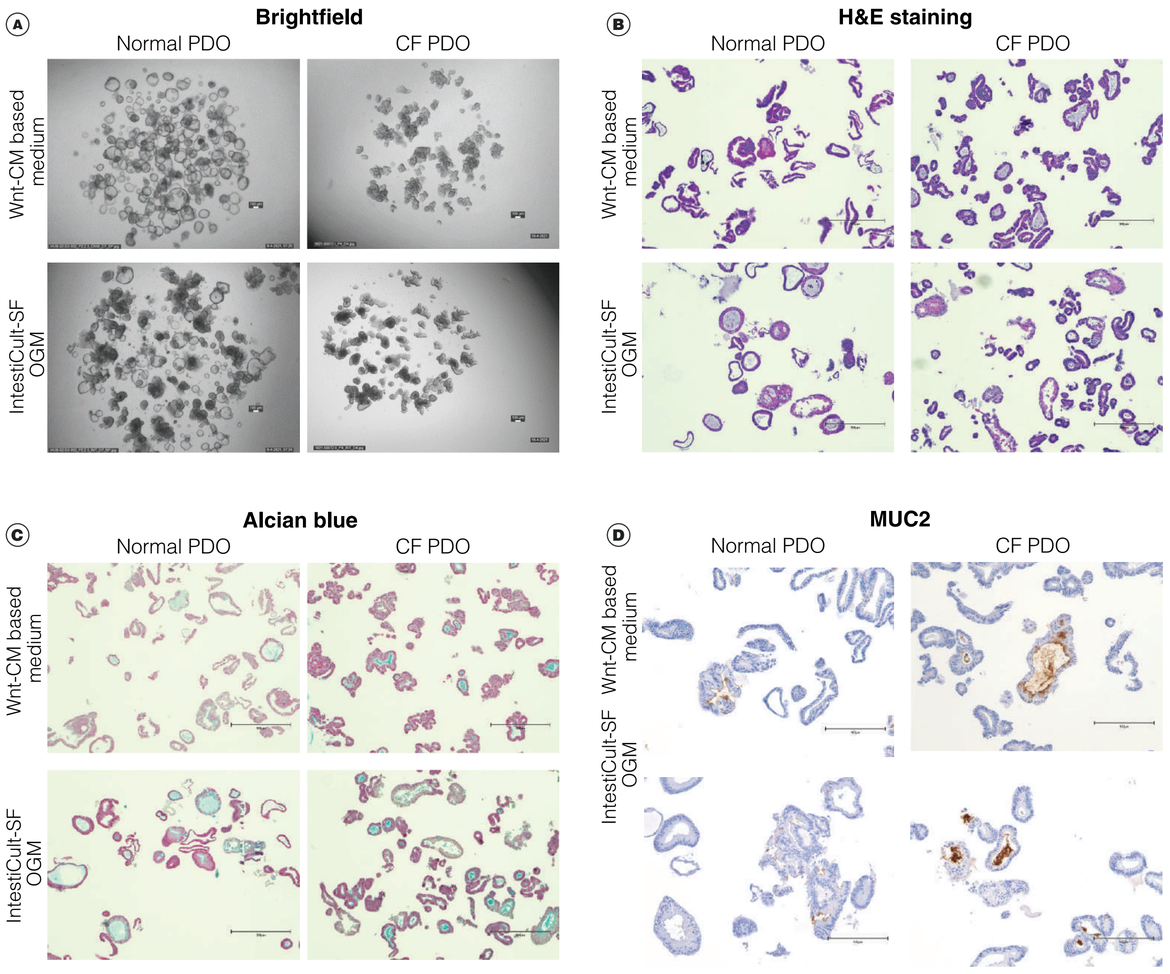


FIGURE 1. Long-Term Expansion of Intestinal Organoids in IntestiCult-SF OGM medium

(A) Frozen CF PDOs were thawed in IntestiCult-SF OGM, and passaged to establish new cryostocks of organoids from IntestiCult-SF OGM cultures. Cryovials containing CF PDOs were shipped and thawed back in IntestiCult-SF OGM. All four organoid lines were cultured in IntestiCult-SF OGM and passaged weekly at an average split ratio of 6.7. The cultures were maintained for a minimum of 10 passages. **(B, C, D, E)** Representative images of CF PDO cultures at passage 1 (top row) and passage 10 (bottom row). The CFTR genetic mutation of each CF PDO is shown on the bottom of the images. Scale bars = 0.5 mm



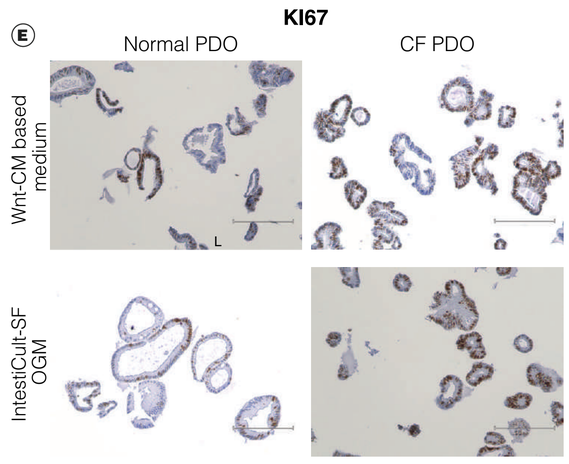


FIGURE 2. Histology staining shows that intrinsic features of normal and CF PDOs are preserved both in Wnt-CM based medium and in IntestiCult-SF OGM.

Three wells of normal PDO or CF PDO maintained in each culture media were pooled and fixed for paraffin embedding. **(A)** Brightfield images of organoid cultures at day 7 shows a slightly more differentiated morphology of organoids in IntestiCult-SF OGM. **(B)** Paraffin sections were stained with Hematoxylin and eosin (H&E). **(C)** Organoids in both media present similar abundance of mucus secreted in the lumen as shown by Alcian blue staining. **(D)** PDOs were stained with antibody against Mucin 2 protein. **(E)** Staining with antibody against KI67 protein shows similar proliferation status of organoids in IntestiCult-SF OGM and in Wnt-CM based media. Scale bars: 100 µm **(A)**, 300 µm **(B, C)**, 150 µm **(D, E)**.

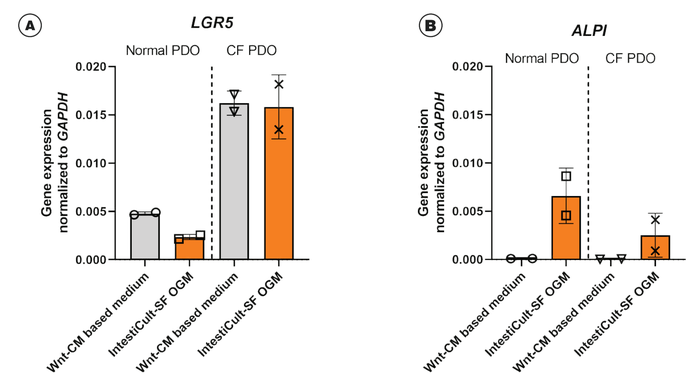


FIGURE 3. q-PCR analysis of normal and CF PDOs cultured in IntestiCult-SF OGM shows elevated expression of differentiation marker compared to those in Wnt-CM based medium.

(A) Stem cell marker *LRG5* is expressed at similar levels in both culture media; **(B)** Upregulation of the enterocyte marker *ALPI* in normal and CF PDOs maintained in IntestiCult-SF OGM medium in comparison to those maintained in Wnt-CM based medium. Bars represent the 2^(-ΔCT) values using GAPDH as a reference gene. CF PDO line has the F508del mutation in both alleles.

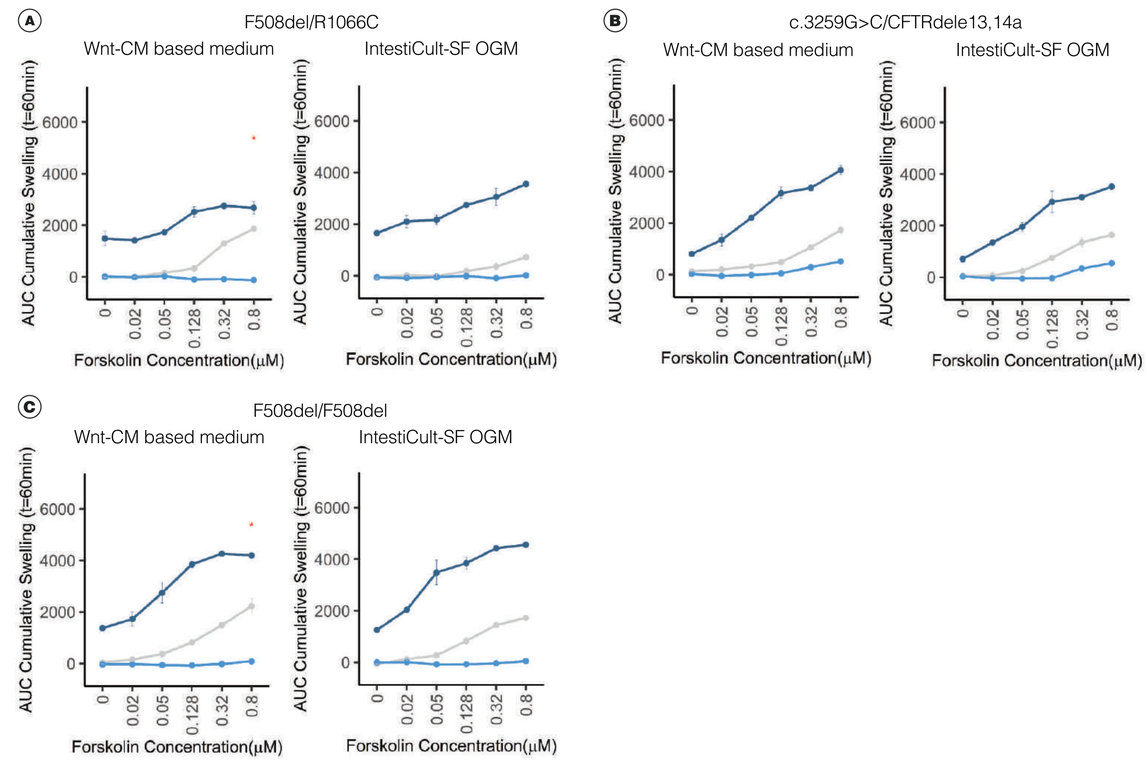


FIGURE 4. CF PDOs cultured and assayed in Wnt-CM based medium and IntestiCult-SF OGM respond similarly to drug treatment in Forskolin-induced swelling assay (FIS).

(A, B, C) The swelling response was quantified by calculating area under the curve (AUC) of relative organoid size over time[1]. The graphs shows the AUC values calculated at 60 min time point. The CFTR genetic mutation of respective CF PDO is shown on top of each graph. After 24 hours of treatment with VX-661 and VX-445 combination, CF PDOs swelled in response to VX-770, and swelled further in response to Forskolin in a dosage-dependent manner. CF PDOs treated with VX-661 alone respond to the VX-770 drug only in the presence of Forskolin at the moderate to high concentration levels.

Summary

- Frozen CF PDOs stocks can be thawed and directly cultured in IntestiCult-SF OGM medium. The cultures can be passaged for at least 10 weeks and additional cryostocks of PDOs can be generated from cultures in IntestiCult-SF OGM
- CF and normal PDOs cultured in IntestiCult-SF OGM displayed similar levels of proliferation compared to Wnt-CM based medium. IntestiCult-SF OGM generated PDO cultures with increased level of mature cells, thus allowing a more balance representation of cellular diversity in organoid cultures
- Functional assay performed in IntestiCult-SF OGM showed similar swelling responses of CF organoids with different mutations compared to Wnt-CM based formulation thus validating IntestiCult-SF OGM for drug testing in patient-derived
- IntestiCult-SF OGM is a commercially available, standardized serum-free and conditioned-medium free formulation that serve as an alternative to lab-made culture medium for Forskolin-Induced Swelling assays and CF colon PDOs

References
- [1] Vonk AM, *et al.* Protocol for application, standardization and validation of the forskolin-induced swelling assay in cystic fibrosis human colon organoids. STAR Protoc. 2020; 1(1):100019

This research was funded in the frame of LSH-TKI project LSHM20088