

Patient derived rectal organoids (PDRO) and 2D monolayers from patient with Cystic Fibrosis

Commonly used acronym: PDRO

Created on: 20-02-2024 - Last modified on: 22-02-2024

Contact person

Anabela Ramalho

Organisation

Name of the organisation Katholieke Universiteit Leuven (KUL)

Department Department of Development and Regeneration

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Education and training, Translational - Applied Research
Type of method	In vitro - Ex vivo
Type of cells/tissues/organs	Intestinal epithelial cells from human rectal biopsies
Specify the type of cells/tissues/organs	Intestinal epithelial cells from human rectal biopsies

DESCRIPTION

Method keywords

Human Intestinal Organoid

3D culture
Transwell
drug repurposing

Scientific area keywords

cystic fibrosis
adult stem cells
epithelial cells
CFTR
Organoid biobank
Disease modeling
intestinal crypt

Method description

Adult intestinal stem cells are grown into closed 3D structures with protruding buds and a luminal compartment. These 3D structures are produced from the stem cells present in the base of crypts isolated from rectal mucosa samples that can be collected by suction or forceps biopsy. The crypts are cultured in extracellular matrix and using a multi-component media that allows fast proliferation of the stem cells as 3D structures; the so-called rectal organoids. This 3D epithelial model maintains disease and patient specific characteristics, thus can be used to evaluate CFTR function, predict responses to modulators, characterized CFTR rare mutations and help in unclear CF diagnoses. Additionally, rectal organoids can be dissociated, cultured as polarized 2D cultures on permeable plastic supports and used to measure transepithelial currents in Ussing chambers.

Lab equipment

- Biosafety cabinet,
- CO2 cell incubator,
- centrifuge,
- microscope.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Organoids are patient-derived primary cells models that faithfully reproduce the subjects' genetic background.
- Organoid cultures can be bio-banked and expanded indefinitely.
- Can be used for personalized testing of responses to CFTR therapeutics as organoid-guided n-of-1' trial approach when traditional clinical trial are not possible due to the reduce number of patients with a specific CF genotype.
- Organoid assays have HTS capability.
- High Levels of CFTR protein in stem cells present in the crypts facilitate testing of efficacy of CFTR therapeutics.
- Isc measurements of polarized 2D cultures on permeable supports may overcome this limitation of ICM with tissues that requires instant analysis without excessive storage times.

Challenges

- Rectal organoids represent only epithelial cells from the intestinal tract present.
- Requires specialized training ECM and medium including growth factors is rather expensive.

Modifications

- Organoids can be cultured from other organs, like lungs.
- Development of more complex models including other cell-types.

Future & Other applications

Organoids biobanked can be used to test novel CF therapies developed in the future like gene therapies.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

- Ramalho AS, Amato F, Gentsch M. Patient-derived cell models for personalized medicine approaches in cystic fibrosis. J Cyst Fibros. 2022 Dec 16:S1569-

1993(22)01419-9. doi: 10.1016/j.jcf.2022.11.007.

- Cuyx S, Ramalho SS, Callebaut I, Cuppens H, Kmit A, Arnauts K, Ferrante M, Verfaillie C, Ensinck M, Carlon MS, Boon M, Proesmans M, Dupont L, De Boeck K, Farinha CM, Vermeulen F, Ramalho AS. Severity of the S1251N allele in cystic fibrosis is affected by the presence of the F508C variant in cis. *J Cyst Fibros*. 2022 Jul;21(4):644-651. doi: 10.1016/j.jcf.2022.05.013.
- Vonk AM, van Mourik P, Ramalho AS, Silva IAL, Statia M, Kruisselbrink E, Suen SWF, Dekkers JF, Vleggaar FP, Houwen RHJ, Mullenders J, Boj SF, Vries R, Amaral MD, de Boeck K, van der Ent CK, Beekman JM. Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis Human Colon Organoids. *STAR Protoc*. 2020 Jun 3;1(1):100019. doi: 10.1016/j.xpro.2020.100019. PMC7580120.
- Cuyx S, Ramalho AS, Corthout N, Fieuw S, Fürstová E, Arnauts K, Ferrante M, Verfaillie C, Munck S, Boon M, Proesmans M, Dupont L, De Boeck K, Vermeulen F; Belgian Organoid Project. Rectal organoid morphology analysis (ROMA) as a promising diagnostic tool in cystic fibrosis. *Thorax*. 2021 Nov;76(11):1146-1149. doi: 10.1136/thoraxjnl-2020-216368.
- Ramalho AS, Fürstová E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, et al. Correction of CFTR function in intestinal organoids to guide treatment of Cystic Fibrosis. *Eur Respir J*. 2020 Sep.

Links

organoids@KULeuven

Coordinated by



Financed by

