Human intestinal enteroids as a model for viral infection

Created on: 26-01-2023 - Last modified on: 15-10-2025

Contact person

Joana Rocha-Pereira

Organisation

Name of the organisation Katholieke Universiteit Leuven (KUL)

Department Microbiology, Immunology & Transplantation

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Human intestinal enteroids derived from human small intestine tissue

DESCRIPTION

Method keywords

organoids

3D culture

Transwell

extracellular matrix

Scientific area keywords

Intestinal organoids

gastroenteric viruses

gastroenteric infection

viral infection

co-culture

Method description

Human Intestinal Organoids (HIOs) are in vitro 3D cell cultures arranged in a crypt-villus structure that incorporate many physiological features of the intestinal epithelium, including the presence of different cell populations (enterocytes, goblet cells, enteroendocrine and Paneth cells). HIOs can be generated from isolated crypts that contained the intestinal stem cells from small intestinal primary tissue (enteroids) or they can be generated from pluripotent stem cells (organoids). HIOs have emerged as a unique opportunity to study hard-to-cultivate enteric viruses in vitro and better understand their biology. We use human small intestine tissue-derived organoids (enteroids) that are 3D cultured in extracellular matrix (ECM, i.e. matrigel) in a growth medium rich in Wnt3, R-spondin and Noggin. For viral infection several approaches can be used a) differentiation of 3D enteroids and infection with virus in suspension; b) seeding of an enteroid single cell suspension in collagen coated plates and infection upon a confluent monolayer or c) seeding of an enteroid single cell suspension in transwell inserts and infection upon a confluent monolayer. Transwells allow co-culture with other cells of interest (e.g. immune cells). The first approach, the 3D infection of enteroids, has been a successful model to evaluate the antiviral activity of compounds and an excellent opportunity to push antiviral drug discovery to the next level.

Lab equipment

- Biosafety cabinet - CO2 cell incubator - Refrigerated centrifuge - Microscope - Micropipettes

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Enteroids preserve the degree and diversity of glycosylation on histo-blood group antigens (HBGAs) of the donor patient. HBGAs are related to the activity of fucosyltransferase 2 gene (FUT2), a crucial genetic factor for susceptibility to some gastroenteric viruses like human norovirus (HuNoV) and rotavirus (HRV). Therefore, enteroids allow for the first time the in vitro replication of some clinical HuNoV and HRV strains.

Challenges

- Intestinal organoids are composed of only epithelial cell types lacking complex mesenchymal heterogeneity and architecture, vasculature, neuronal connections and interaction with immune cells and the intestinal microbial flora. - Organoid culture requires specialized training to manipulate the cells that grow in 3D and the ECM. - Due to the need of ECM and the organoids characteristic’s automatization and high-throughput are difficult. - Medium and related reagents are expensive. - Ethical restrictions and regulations concerning the use of donor material.

Modifications

Triple co-culture of enteroids with fibroblasts and PBMCs-derived macrophages has been performed in a transwell system.

Future & Other applications

- Enteroids are currently being used as a model for antiviral drug discovery. Optimization of methods for higher throughput are ongoing using high-content imaging. - Further interaction with immune cells to study the epithelial immune barrier function in the presence of pathogens and co-culture with other organ-derived organoids will be studied.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Mirabelli C, Santos-Ferreira N, Gillilland MG 3rd, Cieza RJ, Colacino JA, Sexton JZ, Neyts J, Taube S, Rocha-Pereira J, Wobus CE. Human Norovirus Efficiently Replicates in Differentiated 3D-Human Intestinal Enteroids. J Virol. 2022 Nov 23;96(22):e0085522. doi: 10.1128/jvi.00855-22. Epub 2022 Nov 7. PMID: 36342297; PMCID: PMC9683019. Masmoudi F, Santos-Ferreira N, Pajkrt D, Wolthers KC, DeGroot J, Vlaming MLH, Rocha-Pereira J, Buti L. Evaluation of 3D Human Intestinal Organoids as a Platform for EV-A71 Antiviral Drug Discovery. Cells. 2023 Apr 12;12(8):1138. doi: 10.3390/cells12081138. PMID: 37190047; PMCID: PMC10136548. Santos-Ferreira N, Van Dycke J, Chiu W, Neyts J, Matthijnssens J, Rocha-Pereira J. Molnupiravir inhibits human norovirus and rotavirus replication in 3D human intestinal enteroids. Antiviral Res. 2024 Mar;223:105839. doi: 10.1016/j.antiviral.2024.105839. Epub 2024 Feb 17. PMID: 38373532. Santos-Ferreira N, Zhang X, Corneillie L, Van Dycke J, Chiu W, Montpellier C, Neyts J, Cocquerel C, Kaptein S JF, Rocha-Pereira J. Proliferative Cell Targeting and Epithelial Cell Turnover Fuels Hepatitis E Virus Replication in Human Intestinal Enteroids. Gastro Hep Advances, Volume 5, Issue 1, 2026, 100769, ISSN 2772-5723, https://doi.org/10.1016/j.gastha.2025.100769.

Associated documents

jvi.00855-22.pdf

Santos-Ferreira et al, 2026 Gastro Hep advances.pdf

Coordinated by

sciensano

VUB

Financed by

Vlaanderen
verbeelding werkt

bruxelles environnement
leefmilieu brussel
.brussels