Interreg Italia-Österreich
precanmed

SOP-ADSI-4.0	Author:	Issued:
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DIFFERENTIATION OF ORGANOIDS

Purpose

The SOP-ADSI-4.0 was issued the procedure of the differentiation of organoids from colon cancer samples and the respective healthy tissues.

Scope

SOP 1.0 is intended to cover the protocol needed to fully differentiate organoids from fresh tissue of colon cancer samples as well as healthy tissue.

Introduction

For cell culture expansion, intestinal organoids are kept in human intestinal expansion medium. In expansion medium organoids proliferate and consist mainly of stem cells growing in cystic structures (like balloons). For the differentiation into organ-specific cells, the organoids have to be in differentiation medium (see ADSI SOP 1). The removal of several growth factors from the EM initiates the polarization and differentiation into mainly enterocytes, some goblet cells, and enteroendocrine cells. Organoids will become more compact, the cells will polarize and become thicker. The intestinal organoids reach their maximal status of differentiation within 5 to 7 days. After 7 to 8 days, cells will start to undergo apoptosis. Also tumor organoids show the signs of differentiation upon cultivation in DM.

1. Instrumentation, reagents and reagent set-up

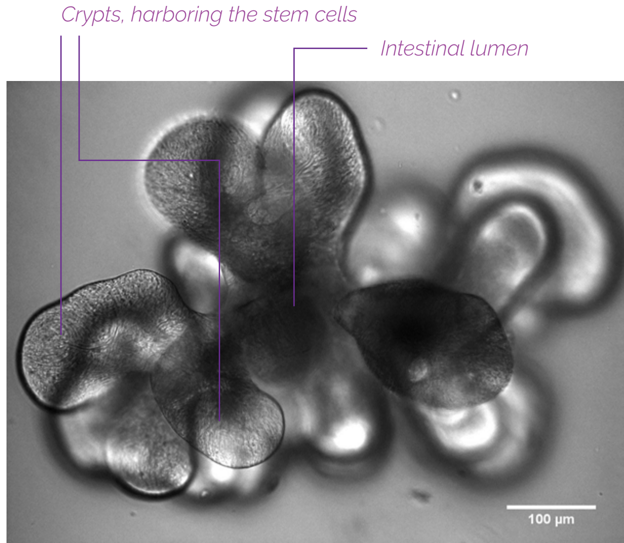

Figure 1 Example image of an intestinal organoid differentiated for approximately 10 days

- Thaw required amount of matrigel on ice, always keep on ice
- 0.25% Trypsin / EDTA, 4˚C
- Put GF- on ice
- Pre-warmed 24 well Culture Plates
- Prepare required culture media
- 15 ml tubes, pre-cooled on ice
- Pipette tips and pipettes (10µl, 200µl, 1000µl)
- Pipette aid and pipettes (10 ml)
- Pre-cool the centrifuge to 4˚C

2. Procedure

2.1 Harvest

- Remove medium from the wells (check if the Matrigel is stable otherwise you might lose the cultures!)
- Add 500 µl ice cold GF- to every well
- Detach Matrigel drop with 1000 µl tip
- Transfer to 15 ml tube (kept on ice)
- Wash wells with 500 µl cold GF- to collect all cells
- Add GF- up to 10-14 ml
- Centrifuge for 3 min 800 rpm at 4˚C
- Remove supernatant (matrigel pellet with the organoids is visible by eye).
- If the matrigel pellet is very big, remove the supernatant and resuspend the pellet with 3ml ice cold GF-. Pipette up and down. Add 7ml of ice cold GF- and centrifuge again for 3 minutes at 1200 rpm at 4˚C

2.2 Trypsinization

- Add 200 to 500 µl 0.25% Trypsin-EDTA (depending on the size of the pellet)
- Pipette up and down
- Incubate 7 min at 37˚C (water bath)
- Pipette up and down with a 100 µl pipette tip (if cells will not get loose either extend the incubation time or attach a 200µl pipette tip to the 1000µl pipette tip)
- Check repeatedly under the microscope (in the tube)
- Repeat incubation cycles until the majority of cells are single cells
- If single-cells still do not appear after 3 times of checking under the microscope, add additional 200 µl 0.25% Trypsin-EDTA and extend incubation cycles at 37˚C

2.3 Seeding

- When single cell suspension is obtained, add 10 ml GF-
- Centrifuge for 5 min, 1500 rpm at 4˚C
- Optional: cell counting
- Remove supernatant
- Resuspend in 200 µl of GF-
- Count cells (in duplicate)

- Calculate how many cells you need (do not seed the single cell too dense! Once differentiated, the organoids will become bigger and form crypts)
- Transfer the required amount to a 15 ml tube
- Add 10ml GF-
- Centrifuge for 3 min at 1500 rpm, 4°C
- Remove the supernatant and resuspend in the required amount GF- (example: 10 µl GF-, 20 µl matrigel per well in 24 well plate) and add matrigel according to the dilution.
- Seed 30 µl droplets per well. Pipette fast to avoid cooling of the pre-warmed plate.
- Incubate for 10-15 min at 37°C (5% CO_2)
- Carefully add 500 µl pre-warmed medium per well.
- Incubate at 37°C (5% CO_2)
- Replace medium every 2 to 3 days with EM
- Let organoids grow for 3-5 days, Organoids become bigger
- After 3-6 days in culture (organoids should not be at maximum size) change medium from EM to DM
- Change medium every 2-3 days
- After 5 days organoids should be fully differentiated, can be kept up to 7 days in DM (Organoids will start to become thicker after about 3 days)
- Organoids can now be used for further processing or experiments (fixation, collection for histological stainings, cryopreservation for RNA/DNA isolation etc…)

3. Applicable references

ADSI-CCO-SOP-1, ADSI-CCO-SOP-2, ADSI-CCO-SOP-3