Title

Cultivation of hCMEC/D3 and hBMEC

Protocol

Cell Seeding:

Materials:

- EBM-2 Medium (Cat. No.: CC-3156, Lonza)
- Phosphate Buffered Saline (PBS) (Catalog Nr: D8537, Sigma Aldrich, Co., stored at 2-8°C)
- Collagen type I coated flask/dish
- Ethanol 70%

Procedure:

1. Take cells out of the liquid nitrogen tank.
2. Put the criovial into the waterbath at 37°C and let the cells thaw until there is no ice left.
3. Warm up medium at 37°C in water bath (ca. 10min) and add the desired volume to a collagen type 1-coated plate.
    1. The bigger the plate, the longer cells will take to grow and to gain confluence.
4. Add medium to the coated flask.
5. Cells can be taken out of the criovial with a pipette and put into the flask.
6. Aspirate liquid up and down to mix cells with medium.
7. Slightly swivel the plate to evenly distribute cells.
8. Check under the microscope if you have cells (small dots).
9. Put flask in the incubator at 37°C.
10. After 1 day at the latest or when cells are attached, medium is aspirated.
    1. Attached cells gain a typical shape: They are elongated and taper to a point at the ends.
11. Wash cells once with PBS to get rid of the DMSO contained in the medium used to freeze cells.
12. Replace with warmed up fresh medium.
13. Cells can be maintained in this flask until they reach confluence.

Cell Cultivation:

Medium in changed every 2-3 days.
Check at each medium change if cells are still alive, how much they have grown and if they are reaching confluence.

Procedure:

1. Check cells under the microscope.
2. Aspirate all the medium.
    1. Aspirate while holding the flask sideways to not touch the surface with the cells and prevent aspirating them.
3. Add new warmed up medium and distribute it evenly over the surface where cells are seeded.

Cell Passaging:

Cells can be passed up to twice a week. Always make sure that the cells are confluent (at least 70%) enough to be passed. 3-4 days after seeding on flasks or petri dishes, cells reach confluence and can be passaged.
Before performing an experiment, passage cells at least once.

Materials:

- Trypsin (Cat. No.: T3924, Sigma-Aldrich)
- Phosphate Buffered Saline (PBS) (Cat. No: D8537, Sigma-Aldrich)
- EBM-2 Medium (Cat. No.: CC-3156, Lonza)

Procedure:

1. Aspirate the medium from your flask.
2. Wash with PBS (use enough to cover the whole surface where the cells are) to get rid of all the medium.
3. Add trypsin.
    1. Cover the whole surface.
    2. Control under the microscope if cells are detaching.
    3. To accelerate the process slightly shake the flask and hit it slightly using your hand. It is also possible to put the cells in the incubator to speed up the procedure.

4. When the cells are detached, add enough medium to inactivate the trypsin.
5. Aspirate up and down to fully inactivate trypsin and to distribute the cells homogeneously.
6. If necessary count cells.
7. Transfer by pipetting the desired amount of cells into one or more new already collagen type I-coated flasks.
    1. If cells are transferred from a small into a bigger flask and are not needed to be used for experiments, they do not need to be counted and all of the cells can be transferred.
    2. Read instruction of further experiment to see how many cells are needed.
8. Add as much medium until you reach the total volume that is needed to each flask.
9. Check under the microscope if you have the cells in the new flask.
10. Incubate cells.

Cell Freezing:

Materials:

- Trypsin (Cat. No.: T3924, Sigma-Aldrich)
- Phosphate Buffered Saline (PBS) (Cat. No: D8537, Sigma-Aldrich)
- EBM-2 Medium (Cat. No.: CC-3156, Lonza)
- DMSO (Cat. No.: A3672, PanReac AppliChem, De)
- Cryo-Tubes

Procedure:

1. Aspirate all the medium from your flask.
2. Wash with PBS (use enough to cover the whole surface where the cells are) to get rid of all the medium.
3. Add trypsin
    1. Cover the whole surface.
    2. Control under the microscope if cells are detaching.
    3. To accelerate the process slightly shake the flask and hit it slightly against your hand. It is also possible to put the cells in the incubator.
4. When the cells are detached, add enough medium to inactivate the trypsin.
5. Aspirate up and down to fully inactivate and to distribute the cells homogeneously.
6. Put $1{,}5*10^6$ - $2*10^6$ cells in every Cryo-Tube. Add 1/10 of DMSO on top.
    1. E.g. 1,5ml cells + 150µl DMSO

8. Move cryovials containing the cells into a container with Isopropanol.
9. Put them into -80°C for at least 1 night up to 2 weeks.
10. Move cryovials into the liquid nitrogen tank.