Video Article

In vitro Differentiation of Mouse Embryonic Stem (mES) Cells Using the Hanging Drop Method

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Abstract

Stem cells have the remarkable potential to develop into many different cell types. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, This promising of science is leading scientists to investigate the possibility of cell-based therapies to treat disease. When culture in suspension without antidifferentiation factors, embryonic stem cells spontaneously differentiate and form three-dimensional multicellular aggregates. These cell aggregates are called embryoid bodies(EB). Hanging drop culture is a widely used EB formation induction method. The rounded bottom of hanging drop allows the aggregation of ES cells which can provide mES cells a good environment for forming EBs. The number of ES cells aggregatied in a hanging drop can be controlled by varying the number of cells in the initial cell suspension to be hung as a drop from the lid of Petri dish. Using this method we can reproducibly form homogeneous EBs from a predetermined number of ES cells.

Video Link

The video component of this article can be found at https://www.jove.com/video/825/

Protocol

1. Gelatinize T75 flask using a 0.1% gelatin solution and incubate plate in a 37°C, 5% CO_2 tissue culture incubator overnight one day before.
2. Take out the mES cells from incubate, aspirate the medium, rinse the ES cell culture with PBS, add 0.05% trypsin solution to coat the bottom of the dish (2 ml/100mm plate).
3. Incubate at 37°C for approximately 1 minutes, until the cells are sloughing off the plate.
4. Gently triturate (pipet up and down) the trypsinized cells four to six times to disperse the ES cells with a plugged Pasteur pipette,. Transfer the dispersed ES cells into a 15-ml conical centrifuge tube containing prewarmed (37°C) mES medium.
5. Collect cells by centrifugation. Aspirate the supernatant, Add 10 ml of mES medium and pipette up and down to form an single cell suspension.
6. Transfer the cell suspension into a T75 flask pre-coated with 0.1% gelatin and incubate at 37°C with 5% CO_2 for one hour
   *After one hour, the fibroblasts have attached to the plate but the stem cells remain in the medium. Pipette up the medium to collect the stem cells.
7. Spin the cells at 1000 rpm for 5 min and aspirate off the mES medium. Then add another 10 ml of mES differentiation medium and resuspend the cells by repetitive pipetting until there appears to be a fine suspension of cells.
8. Count cells with a hemocytometer use differentiation medium to dilute the stem cell suspension to a concentration of 400 to 500 cells per 20ul (20ul/drop) in a sterile basin.
9. Lift lid, carefully invert it and place it on top of the dish containing 10 ml of PBS. Using a multichannel pipette, make rows of 20ul drops on the up-turned inner surface of the lid of the tissue culture dish.
10. Carefully place the dish in the incubator for 2 days. After two days, carefully turn over the plate cover, aspirate 180 ul fresh differentiation medium and put several drops into the well of a 96-well ultralow attachment plate. Then pickup the drop with the pipette and transfer drops, one-by-one, to the 96-well plate. Place the plates into the incubator undisturbed for 3 days.
11. Coat each well of a 48-well tissue culture plate with 300ul of 0.1% gelatin. After adding the gelatin, incubate the plate overnight at 37°C one day ahead before transfer the Ebs.
12. The next day, aspirate the gelatin from the 48-well plate. Then add 300 ul of differentiation medium to each well. Transfer the EBs from the 96 well plates to the 48 well gelatin-coated plates one-by-one. Change the medium the next day and then change the medium every other day to maintain the cells.
13. Spontaneous cardiaomyocyte contractions should be evident within 7 days.

Discussion

The disadvantages of hanging drop method are as follows: the liquid volume of a drop is limited to less than 50ul due to maintaining hanging drops on the lid by surface tension, and it's impossible to change the medium for hanging drops. Observation of forming EBs in drops directly

with microscopy is also very difficult during cultivation. Further more , the hanging drop method consists of two steps, therefore, a series of step of the hanging drop method may be troublesome.

References

1. Wobus, A. M., Wallukat, G., & Hescheler, J. Pluripotent mouse embryonic stem cells are able to differentiate into cardiomyocytes expressing chronotropic responses to adrenergic and cholinergic agents and Ca2+ channel blockers. Differentiation, 48(3), 173-182. (1991).

2. Metzger, J. M., Lin, W. I., & Samuelson, L. C. Transition in cardiac contractile sensitivity to calcium during the in vitro differentiation of mouse embryonic stem cells. J Cell Biol, 126(3), (1994).

