Video Article

Passaging Human Neural Stem Cells

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Abstract

The ability to manipulate human neural stem/precursor cells (hNSPCs) in vitro provides a means to investigate their utility as cell transplants for therapeutic purposes as well as to explore many fundamental processes of human neural development and pathology. This protocol presents a simple method of culturing and passaging hNSPCs in hopes of standardizing this technique and increasing reproducibility of human stem cell research. The hNSPCs we use were isolated from cadaveric postnatal brain cortices by the National Human Neural Stem Cell Resource and grown as adherent cultures on flasks coated with fibronectin (Palmer et al., 2001; Schwartz et al., 2003). We culture our hNSPCs in a DMEM:F12 serum-free media supplemented with EGF, FGF, and PDGF and passage them 1:2 approximately every seven days. Using these conditions, the majority of the cells in the culture maintain a bipolar morphology and express markers of undifferentiated neural stem cells (such as nestin and sox2).

Protocol

Note: For routine culturing of our hNSPCs, we change 50-100% of the media every other day and usually passage them 1:2 once a week. The culture media contains 20% BIT-9500, 1X antibiotic/antimycotic, and growth factors (EGF, FGF, and PDGF each at 40 ng/ml) in DMEM:F12 base media.

Preparing the Coated Flask and Dissociating the Cells

1. To prepare new flasks for the passaged cells, coat T25 flasks with 10 µg/ml human fibronectin in EMEM for 4 hours, or overnight, in a 37°C tissue culture incubator. Right before passaging the cells, remove the fibronectin solution and rinse the flasks with PBS.
2. Transfer the old "conditioned" media from the cells to be passaged to the new fibronectin-coated flasks (such that half the final volume of media for each flask will be the "conditioned" media). These culturing conditions help keep these cells in their undifferentiated state.
3. Once all of the media is removed from the cells to be passaged, rinse the cells once with PBS. Take care to not dry out the cells.
4. Add Cell Dissociation Buffer (CDB) directly onto the cells (1.0-1.5 ml for a T25 flask). Incubate cells in the buffer for about 5 minutes at room temperature. Gently tapping the flask will help speed up cell detachment.

Centrifuging, Resuspending, and Plating Cells

1. Add serum-containing media (DMEM:F12 with 10% heat-inactivated fetal bovine serum) (use ~3X the volume of CDB) to the flask with detached cells. Remove the media and cells to a 15 ml conical tube. Use a small volume of fresh serum-containing media to rinse the flask and recover residual cells.
2. Centrifuge the media and cells at 1000 rpm (~200xg) for 5 minutes.
3. Suction off the serum-containing media and resuspend the cells in fresh culture media, pipetting up and down to make a homogeneous cell suspension.
4. Transfer half of the resuspended cells into each new flask for a 1:2 split. Note the new passage number on the flask.

Discussion

We have found that this protocol provides reliable cultures of hNSPCs. One critical factor for our cells is that they need to be kept as fairly dense cultures and cannot be passaged to the point that the cells are sparse. In our hands, sparse cultures grow very slowly or completely cease dividing. For this reason, we usually split our cultures 1:2 or 1:3 when a culture is extremely dense. Coating the surface of a culture dish with fibronectin is important since it promotes good cell attachment and migration, but, unlike laminin, it also permits the use of Cell Dissociation Buffer (CDB) to detach cells. Cell attachment to laminin is too strong and requires the use of a proteolytic enzyme (e.g. trypsin) for cell detachment. Non-enzymatic CDB is preferred to trypsin since proteolytic cleavage of cell surface proteins may lead to morphological changes in hNSPCs over time. Also, culturing hNSCPs in conditioned media helps maintain these cells in their undifferentiated state.

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References

1. Flanagan, L.A., L.M. Rebaza, S. Derzic, P.H. Schwartz, E.S. Monuki. Regulation of human neural precursor cells by laminin and integrins. J. Neurosci. Res. 83(5): 845-856, 2006.

2. Nethercott, H.N., H. Maxwell, P.H. Schwartz. Neural stem cell culture. In Human Stem Cell Manual: A Laboratory Guide. J.F. Loring, R.W. Wesselschmidt, and P.H. Schwartz, eds. Elsevier/Cell Press, 2007.

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3. Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, Gage FH. Cell culture. Progenitor cells from human brain after death. Nature. 411(6833): 42-43, 2001.

4. Schwartz PH, Bryant PJ, Fuja TJ, Su H, O'Dowd DK, Klassen H. Isolation and characterization of neural progenitor cells from post-mortem human cortex. J Neurosci Res. 74(6): 838-851, 2003.

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