Primary Culture of SVZ-derived Progenitors Grown as Neurospheres

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[Abstract] SVZ-derived progenitors grown as neurospheres is a well-known model to study neural stem cell and progenitor functions such as proliferation, differentiation/self-renewal, and migration (Durbec and Rougon, 2001). This protocol is for preparing a culture of SVZ-derived progenitors from 8 early postnatal mouse brains (P0 to P3). One week after cell plating, we can observe round floating neurospheres, each resulting from the clonal expansion of a single EGF/FGF responsive neural progenitor.

Materials and Reagents

1. New born mice
2. Phosphate Buffered Saline (PBS) (Life Technologies, catalog number: 14040-091)
3. Hank's Balanced Salt Solution (HBSS) (Life Technologies, catalog number: 14170-088)
4. Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, catalog number: 61965-026)
5. Ham’s F-12 nutrient mix (F12) (Life Technologies, catalog number: 31765-027)
6. Trypsin (Sigma-Aldrich, catalog number: T5266)
7. Insulin (Sigma-Aldrich, catalog number: I1882)
8. Holo-transferrin (Sigma-Aldrich, catalog number: T0665)
9. Putrescine (Sigma-Aldrich, catalog number: P5780)
10. Progesterone (Sigma-Aldrich, catalog number: P8783)
11. Sodium selenite (Selenium) (Sigma-Aldrich, catalog number: S5261)
12. Penicillin-streptomycin (Life Technologies, catalog number: 15140-130)
13. Fetal Bovine Serum (FBS) (Life Technologies, catalog number: 10106-169)
14. B27 (Life Technologies, catalog number: 17504-044)
15. rhFGFbasic (Peprotech, catalog number: 167100-18B-B)
16. rhEGF (Peprotech, catalog number: 167AF-100-15A)
17. Neurospheres defined medium (see Recipes)
18. Trypsin (see Recipes)

Equipment

1. Sterilin Universal Container (Thermo Fisher scientific, catalog number: 128A/P)
2. Standard TC BD Falcon 60 mm cell culture dish (BD Biosciences, Falcon®, catalog number: 353002)
3. Vibratom (Microm, model: HM450)
4. Stereoscopic Microscope
5. Cell culture Hood and Incubator
6. Scissors, fine forceps, spatula, micro knives
7. Ice bucket
8. Fire-polished glass Pasteur pipettes
9. Water bath

Procedure

1. Dissect the brain from new born mice.
2. Cut 400 µm thick coronal sections of the brain in ice cold PBS with a Vibratom (Figure 1A) (from the olfactory bulb to the anterior horn of the lateral ventricle). Keep the first two sections of each brain containing the lateral wall of the lateral ventricles and place them in ice cold HBSS.
3. Under the microscope, dissect out the lateral walls of the lateral ventricles (Figure 1B). Take a forceps with one hand to handle slice and keep it on the bottom of the dish, and a micro knife with the other to cut out the zone of interest. Discard the rest of the slice after each dissection.
4. Cut the tissues into small cubes (400 µm cubes) using sterile micro knives.
5. Pipette the tissues within a volume of 800 µl HBSS and place them in a 30 ml Sterilin Universal Container. Add 200 µl of trypsin 12.5 mg/ml. Incubate 5 min at 37 °C in a water bath.
6. Add 10 volumes of 10% HBSS-FBS. Using fire-polished glass Pasteur pipettes, gently pipet up and down to help the dissociation. Take of a droplet regularly and check under the microscope whether the dissociation is complete.
7. Centrifuge at 800 *x g* for 7 min at room temperature (RT), discard the supernatant, and resuspend the pellet in 10 ml of fresh HBSS. Take of a sample and count the number of cells.
8. Centrifuge the cell suspension at 800 *x g* for 7 min at RT, discard the supernatant, and resuspend in Standard TC BD Falcon 60 mm cell culture dishes at the concentration of

25,000 cells/ml in neurospheres defined medium, supplemented with 2% B27, bFGF (20 ng/ml) and EGF (20 ng/ml).

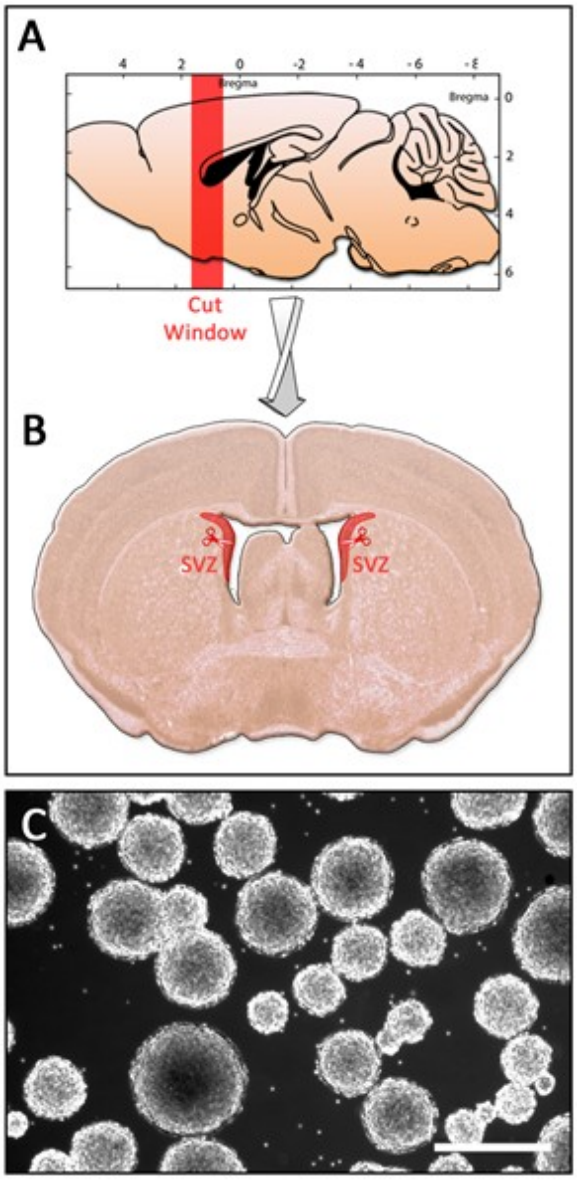

Figure 1. Dissection of the neonatal mouse SubVentricular Zone. A. Illustration of a newborn mice sagittal section showing the cut window when using the vibratome. B. Coronal section showing parts to dissect to isolate lateral ventricules' tissues. C. Neurospheres obtained after 1 week long culture. Scale bar, 500 µm.

9. Every 3 days, add a half-volume of doubly-supplemented [4% B27, bFGF (40 ng/ml) and EGF (40 ng/ml)] neurospheres defined medium. To avoid any sphere attachment to the bottom of the culture dish, don't extend the culture beyond 1 week (Figure 1C).

Recipes

1. Neurospheres defined medium
   DMEM/F12, 3:1 volumes respectively
   5 g/ml Insulin
   100 g/ml Holo-transferrin
   100 M Putrescine
   20 nM Progesterone
   30 nM Selenium
   1% Penicillin-streptomycin (100 IU/ml and 100 g/ml, respectively)
2. Trypsin (prepare each time a new aliquot)
   Dissolve 12.5 mg of Trypsin in 1 ml of ice cold HBSS

Acknowledgments

This protocol is adapted from Durbec and Rougon (2001) and Vernerey *et al*. (2013).

References

1. Durbec, P. and Rougon, G. (2001). Transplantation of mammalian olfactory progenitors into chick hosts reveals migration and differentiation potentials dependent on cell commitment. *Mol Cell Neurosci* 17(3): 561-576.
2. Vernerey, J., Macchi, M., Magalon, K., Cayre, M. and Durbec, P. (2013). Ciliary neurotrophic factor controls progenitor migration during remyelination in the adult rodent brain. *J Neurosci* 33(7): 3240-3250.

