Video Article

Culture of Mouse Neural Stem Cell Precursors

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Abstract

Primary neural stem cell cultures are useful for studying the mechanisms underlying central nervous system development. Stem cell research will increase our understanding of the nervous system and may allow us to develop treatments for currently incurable brain diseases and injuries. In addition, stem cells should be used for stem cell research aimed at the detailed study of mechanisms of neural differentiation and transdifferentiation and the genetic and environmental signals that direct the specialization of the cells into particular cell types. This video demonstrates a technique used to disaggregate cells from the embryonic day 12.5 mouse dorsal forebrain. The dissection procedure includes harvesting E12.5 mouse embryos from the uterus, removing the "skin" with fine dissecting forceps and finally isolating pieces of cerebral cortex. Following the dissection, the tissue is digested and mechanically dissociated. The resuspended dissociated cells are then cultured in "stem cell" media that favors growth of neural stem cells.

Protocol

1. Mouse neural precursors (NPCs) were isolated from E12.5 embryo cortex.
2. Skin and mesenchymal layers were removed from dissected telencephalic vesicles.
3. Vesicles were incubated in 0.05% trypsin with 0.02% EDTA and 0.2% BSA in HBSS for 20 minutes at 37°C.
4. Trypsinization was stopped by an equal volume of 1 mg/ml soybean trypsin inhibitor (Sigma #T6522) in HBSS.
5. Tissue digests were dissociated using several rounds of trituration with fire-polished Pasteur pipettes.
6. Cells were washed once with 0.2% BSA in HBSS and plated at 50,000 cells/ml on laminin-coated coverslips in media with 20 ng/ml EGF, 10 ng/ml FGF2 (R&D Systems or Peprotech), and 2 ug/ml heparin (Sigma).

Discussion

Great advances in our understanding of CNS development and stem cell biology have been made possible by our ability to harvest, isolate and culture embryonic neural stem cells. This video demonstrates the dissection of E12.5 mouse cerebral cortex and the subsequent disaggregation and culturing of embryonic neural stem cells. Many other other similar methods have been successfully employed by other investigators.

References

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