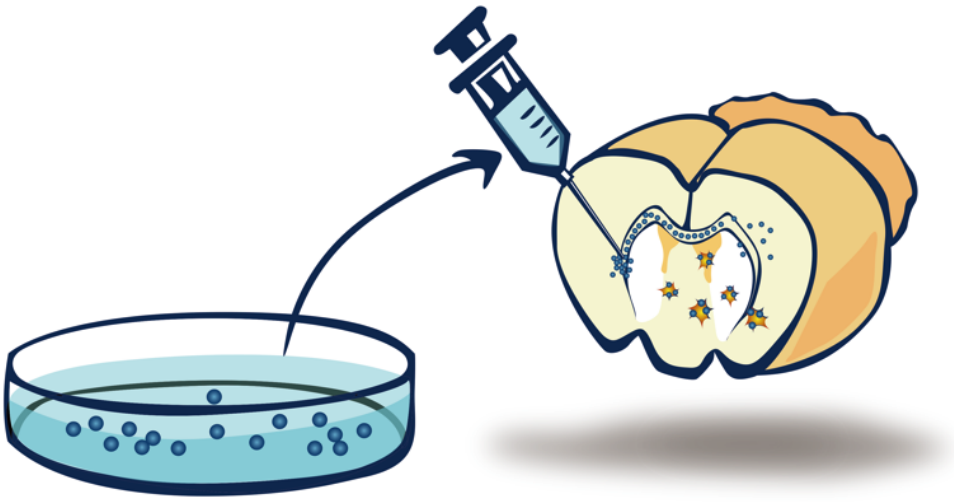
Experts' Protocol Series

Transplantation of Axol™ hNPCs into neonatal rat cerebral cortex



Application Protocol
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I. INTRODUCTION

Cell transplantation is a commonly performed technique in neuroscience research and a number of methods and approaches can be used depending on research interests. Variability in survival and integration of grafted cells is an issue in transplantation research. The immune response to any type of transplant is a key consideration therefore many investigators treat transplanted animals with immunosuppressant drugs or use an immunocompromised host e.g. Nude rats to avoid immune-mediated cell death of the grafted cells. In the case that transplantation into an adult host is unnecessary, grafting can be performed in neonatal hosts prior to the full development of the immune response to avoid immunosuppression. **This protocol describes how perform transplantation of Axol™ hNPCs into a neonatal (day 0-2) rat host.**

II. MATERIALS REQUIRED

Culturing and Differentiation of Axol™ hNPCs

- Please refer to the "Culture and Differentiation of Axol™ hNPCs" instruction manual for full description of cell preparation and required materials.

  Note: Axol™ hNPCs should ideally be grown in culture for 7-8 days after thawing for this procedure.

- D-PBS
- Conical tube (15mL)
- Eppendorf tubes
- Axol™ Neural Maintenance Medium (**ax0031a&b**)
- Axol Neural Unlock™ (**ax0044**)
- Axol Sure Boost™ (**ax0045**)
- Haemocytometer
- Vertical laminar flow hood certified for Level II handling of biological materials
- Incubator with humidity and gas control to maintain 37°C and >90% humidity in an atmosphere of 5% CO_2 in air
- Tissue culture centrifuge
- Inverted microscope with 4X, 10X and 20X objectives.

Transplantation of Axol™ hNPCs

- Axol™ hNPCs resuspended in a concentration of 50,000-100,000 cells per µL
- Ice bucket filled with wet ice
- Hamilton syringe with a 30 gauge needle (Custom made. Other sizes may work, but would need to be optimised)
- 26 gauge needle
- Black fine-tip marker
- Timer
- Blue-tack or equivalent material that can easily be moulded into shape
- Platform to hold pip for visualisation under microscope and injection. (Platforms can be made from 2-3 100mm cell culture dishes filled with ice. Blue tack in the shape of a head holder/stabiliser is placed on top of the platform)
- Transfer cage
- Recovery cage with heat source (heat blanket can be placed under the cage)
- Stereotaxic frame with needle/syringe holder
- Surgical microscope
- Side lamps for surgical illumination (if not provided with microscope)

III. PROCEDURES

Collection of Axol™ hNPCs for Transplantation

Please refer to the "*Culture and Differentiation of Axol™ hNPCs*" instruction manual for full description. Modified from Passaging Axol™ hNPCs.

1. Discard the complete medium from the culture vessel.

2. Gently rinse the surface of the cell layer with D-PBS (2mL D-PBS per 10cm^2) by adding the D-PBS to the side of the vessel and rocking back and forth a few times.

3. To detach the cells, add pre-warmed **Axol Neural Unlock™** (1mL per 10cm^2 culture surface area). Incubate the cells for 3 to 5 minutes at **37°C**.

4. Once the cells are detached, gently pipette the cells up and down to break the cell clumps into a single cell suspension.

5. Stop the dissociation reaction by adding 4 volumes of **Complete Axol Neural Maintenance Medium** (e.g. if 1mL **Axol Neural Unlock™** is used, then add 4mL medium to stop the enzymatic action).

6. Transfer the cells to a 15mL conical tube and centrifuge the tube at **200g** for 5 minutes at room temperature. Discard the supernatant.

7. Resuspend the cells In 1mL Axol Neural Maintenance Medium and count cells using a haemocytometer. After counting the cells, centrifuge the tube at **200g** for 5 minutes at room temperature.

8. Resuspend the cells in the desired amount of Axol Neural Maintenance Medium supplemented with 1000X **Axol Sure Boost™** stock solution i.e. 1µL **Axol Sure Boost™** per 1mL medium. Cells should be kept warm at **37°C** if possible or room temperature for shorter periods of time (2-3 hours maximum) is also adequate.

NOTE: Optimal concentration for cell transplantation is 50,000-100,000 cells/µL.

Neonatal Transplantation Procedure

1. Remove entire litter from mother and home cage and place into a transfer cage. Also take some of the original bedding from the home cage and place into the transfer cage to continue the same smell and surroundings for the pups. This will prevent the mother from rejecting the pups when they are returned to the home cage.

2. Anaesthetise first animal on ice for approximately **5 minutes**. Once the animal is anaesthetised, transfer animal to platform (containing ice to maintain hypothermic anaesthesia).

   Note: Hypothermia is highly recommended as the method of anaesthesia in this protocol. Inhalant anaesthesia can be used, but is highly variable/unreliable at this age due to the breathing pattern of neonates. Injectable anaesthesia is not recommended as it has been known to have a high mortality rate at this age. If unfamiliar with these procedures, check with local/national regulations and seek veterinary advice.

3. Position platform within stereotaxic frame and under microscope for visualisation of the head of the animal.

   Note: Animals of this age cannot be secured into the frame with adult ear bars so care must be taken to ensure the platform is secured into place and doesn't move.

4. Fill Hamilton syringe (with the 30 gauge needle) with the desired amount of cells, taking care not to clog the tip of the needle. Reposition the needle in the needle holder held in the stereotaxic frame.

   Note: 50,000 – 100,000 cells/µL in a 30 gauge needle is the smallest size that should be used for best results.

5. Find bregma by visualisation through the skin (albino rats are the easiest for visualisation). Make a small mark on bregma with a black marker.

6. Position your needle to be centred on bregma and proceed to finding your desired co-ordinates by manipulating the needle holder to desired injection area.

   Note: Co-ordinates will vary depending on your intended injection site. Consult appropriate brain atlases or published literature for more information. For the presumed sensorimotor cortex of P0 pups, the following co-ordinates can be used for two unilateral injections:- 1.0mm AP, 1.0mm ML, -0.5mm DV; -0.5mm AP, 1.0mm ML, -0.5MM DV.

7. Before you inject, it helps to first insert a 26 gauge needle into the desired injection site to open up an injection hole in the skull. Once this is done, the injection needle can be inserted. Inject approximately 0.5-1µL/site, or less if using several injection sites. **Check with local/national regulations for injection volume limitations.**

8. Inject manually over the course of 1 minute per injection, followed by a wait period of 2 minutes before needle withdrawal. Repeat for desired number of injections.

   Note: An injection pump can be used if desired, but keep injection times to a minimum since hypothermia anaesthesia is used. For longer injection times, consider using inhalation anaesthetics.

9. Once injections are complete, transfer animal to a warm recovery cage, with bedding from the original cage to ensure home cage smells are maintained and risk of rejection by the mother is minimised.

10. Repeat for entire litter. Once procedure is running smoothly, consider anaesthetising subsequent animals while injecting another animal. Ensure that the timing is observed so that the initial hypothermia **doesn't exceed 5-6 minutes** prior to the procedure. In total, the entire anaesthesia session **should not exceed 10-14 minutes** for the best outcome.

11. It is difficult to individually mark animals for unique identification at this age, also in keeping with minimal disturbance of the pups to prevent rejection by the mother. Tail marking is not effective as the mother will clean the animal of all markings within 24 hours. Ear clipping is possible, but not preferable as this may stress the mother.

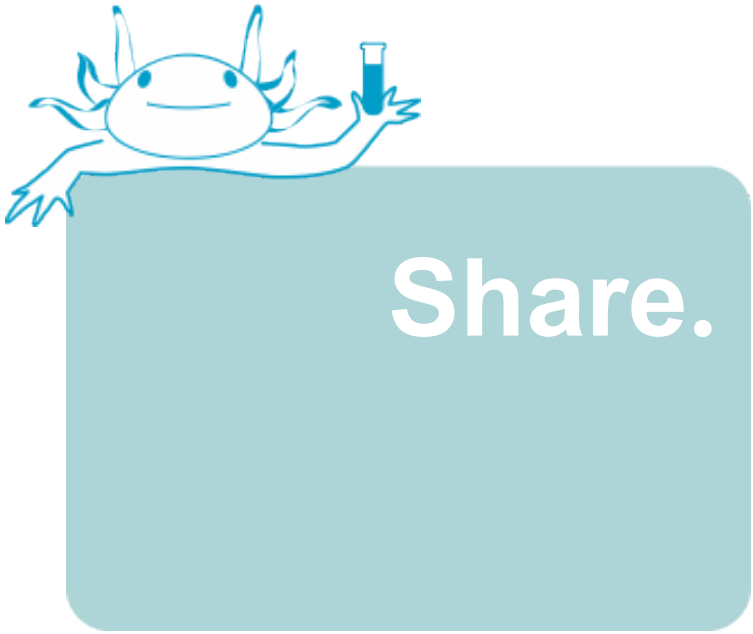
IV. ANTICIPATED RESULTS

- Cell survival of Axol™ hNPCs into neonatal hosts is expected to be high at 1 month post-transplantation.
- Survival beyond 1 month post-transplantation is also expected, however there may be a gradual decrease over time.
- Implanted cells can be identified using human specific antibodies e.g. the human nuclear marker HuNuclei (Millipore cat no: MAB1281), or other markers as described in the *Culture and Differentiation of Axol™ hNPCs* Instruction Manual.

Notes:

For more application protocols, please visit:

axolbio.com/application-protocols



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