Video Article

Hypoxic Preconditioning of Marrow-derived Progenitor Cells As a Source for the Generation of Mature Schwann Cells

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Abstract

This manuscript describes a means to enrich for neural progenitors from the marrow stromal cell (MSC) population and thereafter to direct them to the mature Schwann cell fate. We subjected rat and human MSCs to transient hypoxic conditions (1% oxygen for 16 h) followed by expansion as neurospheres upon low-attachment substratum with epidermal growth factor (EGF)/basic fibroblast growth factor (bFGF) supplementation. Neurospheres were seeded onto poly-D-lysine/laminin-coated tissue culture plastic and cultured in a gliogenic cocktail containing β-Heregulin, bFGF, and platelet-derived growth factor (PDGF) to generate Schwann cell-like cells (SCLCs). SCLCs were directed to fate commitment via coculture for 2 weeks with purified dorsal root ganglia (DRG) neurons obtained from E14-15 pregnant Sprague Dawley rats. Mature Schwann cells demonstrate persistence in S100β/p75 expression and can form myelin segments. Cells generated in this manner have potential applications in autologous cell transplantation following spinal cord injury, as well as in disease modeling.

Video Link

The video component of this article can be found at https://www.jove.com/video/55794/

Introduction

The transplantation of neural progenitors and their derivatives demonstrates promise as a treatment strategy following traumatic nerve injury[1,2] and neurodegeneration[3,4]. Prior to clinical application, it is essential to ensure: i) a method for accessing and expanding upon an autologous source of stem/progenitor cells and ii) a means to direct them to relevant, mature cell types[3]. Our interest in cell therapy for spinal cord injury led us to seek a robust, autologous cell source of neural progenitors from adult tissues.

A subpopulation of MSCs originates from the neural crest and is readily accessible from the marrow cavity. These cells are neural progenitors that can generate neurons and glia[5]. Animal models of cerebral ischemia demonstrate that hypoxia promotes the proliferation and multipotency of neural progenitors within the brain[6]. This was the basis for utilizing hypoxic preconditioning as a means of expanding upon marrow-derived neural progenitors.

The transplantation of Schwann cells into the injured spinal cord promotes regeneration[2]. SCLCs can be generated from MSCs by means of supplementation with gliogenic factors (*i.e.,* β-Heregulin, bFGF, and PDGF-AA) but demonstrate phenotypic instability. Upon the withdrawal of growth factors, they revert to a fibroblast-like phenotype[7]. Phenotypic instability is undesirable in cell transplantation due to the risk of aberrant differentiation and carcinogenesis. As Schwann cell precursors are associated with axon bundles within the embryonic peripheral nerve[8], we were led to coculture SCLCs with purified embryonic DRG neurons[7,9]. Resultant mature Schwann cells are fate-committed and demonstrate function *in vitro*[7,9] and *in vivo*[10].

Our protocol for the enrichment of neural progenitors from MSCs is simple and efficient and results in an increase in cell number for subsequent assays. The derivation of fate-committed Schwann cells via the coculture platform allows for the study of glial differentiation and for the generation of stable and functional Schwann cells for potential clinical application.

Protocol

All procedures involving animals were performed in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Committee on Use of Live Animals for Teaching and Research, Li Ka Shing Faculty of Medicine, The University of Hong Kong. Human bone marrow samples were obtained from the iliac crest of healthy donors after obtaining informed consent. Protocols were approved by the Institutional Review Board, The University of Hong Kong.

1. Preparation of Rat MSC Cultures

1. **Harvest of MSCs from the femur**
   1. Autoclave all dissection tools (*i.e.,* fine dissecting scissors, blunt-tipped cutting scissors, and toothed forceps) at 180 °C for at least 2 h prior to use.
   2. Prepare MSC growth medium comprising of minimal essential medium - alpha modification (αMEM) supplemented with 15% fetal bovine serum (FBS) and penicillin/streptomycin (P/S, 1% v/v).
   3. Sacrifice young male Sprague Dawley rats (200-250 g bodyweight) by pentobarbitone overdose (240 mg/kg bodyweight, intraperitoneal).
      NOTE: Marrow samples from different rats should be processed separately.
   4. Place the sacrificed animals in supine position. Clean their abdomen and lower limbs thoroughly with 70% ethanol.
   5. Remove the skin and subcutaneous tissue over the medial thighs using fine dissecting scissors and forceps. Remove the thigh muscles circumferentially until the femur is exposed. Continue this proximally and distally until the knee and hip joints are seen. Disarticulate the femur through the hip and knee joint using blunt-tipped cutting scissors.
      NOTE: Do not transect the femur to expose the marrow cavity at this stage. Transfer intact femurs to a laminar flow tissue culture hood for further processing.
   6. Use blunt tipped cutting scissors to transect the distal and proximal ends of the femur through the metaphysis.
   7. Place a 70 μm cell strainer over a 50 mL conical tube. Insert a 21 G, 10 mL syringe containing phosphate-buffered saline (PBS, 10 mM Na_2HPO_4, pH 7.4) into the exposed femoral canal and flush the marrow content into the conical tube by repeated flushing.
      NOTE: Approximately 20 mL of PBS is used to flush each femur. If the color of the flushed content remains blood-stained and turbid, a larger volume can be used.
   8. Collect the cells by centrifugation at 480 x g for 5 min. Discard the supernatant. Resuspend the cell pellet in 10 mL of MSC growth medium. Plate the cells onto a 10 cm tissue culture dish. Place the tissue culture dishes into a cell incubator (37 °C, 5% CO_2). Record the initial day of plating as day 0.
      NOTE: In all steps involving centrifugation, set the brake for maximal deceleration.

2. **Establishment and expansion of MSC colonies**
   NOTE: This protocol relies on tissue culture plastic adherence as a means to select for MSCs from within the marrow cavity[11]. The bone marrow content is allowed to adhere to the tissue culture plastic for 2 days.
   1. On day 2, rinse the culture plates three times with 10 mL of PBS to remove non-adherent cells. Replace the PBS with 10 mL of MSC growth medium after rinsing. Wash the cells with PBS and replenish with MSC growth medium every 3 days.
      NOTE: MSC colonies should be visible by day 6-7 (**Figure 2A**).
   2. Passage the cells by day 10 by removing the growth medium and rinsing the cells with PBS. Add 1.5 mL of recombinant enzymatic cell dissociation reagent and incubate at 37 °C for 5 min. Add 3 mL of MSC growth medium to neutralize the reaction. Collect the detached cells by centrifugation at 250 x g for 5 min.
   3. Quantify the cells within the pellet using a hemocytometer after an appropriate dilution in PBS.
   4. Seed passaged cells at a density of 40,000 cells/cm² into a 10-cm culture plate in MSC growth medium.
      NOTE: Rat MSCs should reach 80-90% confluence within 2 days of passaging (**Figure 2B**). Cells can be passaged as described in step 1.2.2 for up to 8 passages. MSCs can be characterized by means of immunocytochemistry and their capacity for trilineage differentiation (**Figure 3**)[12]. Only MSC cultures between passages 3 and 8 are subject to subsequent hypoxic preconditioning and neural progenitor enrichment. MSCs of greater passage number adopt a flattened morphology (**Figure 2C**) and do not produce sufficient numbers of neural progenitors. These cultures should be discarded.

2. Preparation of Human BMSC Cultures

1. Dilute 1 mL of human bone marrow aspirate with 9 mL of MSC growth medium and plate the cells on a 10-cm tissue culture dish. Maintain the cultures in a cell incubator (37 °C, 5% CO_2).
2. Remove the medium after 2 days and gently rinse the cultures three times with 10 mL of PBS to remove non-adherent cells. After the final rinse, remove the PBS and replace it with 10 mL of MSC growth medium. Replenish the growth medium after every 3 days of culture after the PBS rinse.
   NOTE: MSC colonies should be visible by day 6-7. The number of colonies may vary between subjects.
3. Passage the cells on day 10, as described in step 1.2.2. Quantify the cells within the pellet using a hemocytometer after an appropriate dilution in PBS. Seed the passaged cells at a density of 40,000 cells/cm² onto a 10 cm culture plate in MSC growth medium.
   NOTE: Human MSCs (**Figure 2D**) demonstrate a similar morphology to rat MSCs and likewise should reach 80-90% confluence within 2 days of passaging. They should be characterized by means of immunocytochemistry and their capacity for trilineage differentiation[12]. As with rat MSCs, human MSCs that are between passage 3 and 8 are subject to subsequent hypoxic preconditioning and neural progenitor enrichment.

3. Hypoxic Preconditioning

1. Disassemble the hypoxia chamber components (*i.e.,* base, lid, trays) after releasing the ring clamp and wipe the individual parts clean with 70% ethanol. Place the chamber components within a laminar flow tissue culture hood for sterilization under UV light for 15 min.
2. Prior to hypoxic preconditioning, remove the medium and rinse the rat and human MSC cultures (sections 1 and 2) with 10 mL of PBS. Replace the PBS with 10 mL of MSC growth medium supplemented with 25 mM HEPES.
   NOTE: The MSCs cultured upon 10 cm dishes should have reached 80-90% confluency prior to be being subject to hypoxic preconditioning.
3. Place the culture dishes within the hypoxia chamber. Reassemble the chamber components and tighten the ring clamp. Flush a gas mixture of 99% N_2/1% O_2 into the chamber at a flow rate of 10 L/min for 5 min.

4. Seal the connecting ends of the hypoxia chamber to ensure there is no gas leakage. Place the chamber inside the cell incubator (37 °C, 5% CO_2) for 16 h.
5. Upon completion of the hypoxic preconditioning, remove the cultures from the chamber in preparation for the subsequent neural progenitor enrichment culture.

4. Neural Progenitor Enrichment Culture

1. Prepare neural progenitor medium comprised of Dulbecco's modified eagle medium/Ham's nutrient mixture F12 (DMEM/F12) supplemented with B27 (2% v/v), basic fibroblast growth factor (bFGF, 20 ng/mL), epidermal growth factor (EGF, 20 ng/mL), and P/S (1% v/v).
2. Detach the hypoxic preconditioned rat/human MSCs, as described in step 1.2.2. Collect the detached cells by centrifugation at 250 x g for 5 min. Quantify the cells within the pellet using a hemocytometer after appropriate dilution in PBS.
3. Resuspend the cells in neural progenitor medium and seed on low-attachment, 6-well plates at a density of 6,000 cells/cm^{2}. Place the culture in a cell incubator (37 °C, 5% CO_2) for 12 days. Replenish 75% of the neural progenitor medium every 3 days.
    NOTE: Sizeable non-adherent cell clusters should be observed by day 6-7. By day 10-12, neurospheres with a diameter ≥ 100 µm can be observed (**Figure 4**). Hypoxic preconditioned MSCs should yield more neurospheres as compared to MSCs cultured under normoxic conditions[9].
4. Collect neurospheres on day 12 by aspirating them into a 10-mL pipette and transferring them to a 15 mL conical tube. Centrifuge the neurospheres at 250 x g for 5 min.
    NOTE: Neurospheres can be characterized on day 12 for neural progenitor markers, such as nestin and GFAP[7].

5. Generation of Fate-committed Schwann Cells via Coculture with DRG Neurons

1. **Preparation of purified rat DRG neurons**
    1. Autoclave all dissection tools (*i.e.,* dissection scissors, forceps, two microdissection forceps, and microdissecting scissors) at 180 °C for at least 2 h prior to use.
    2. Coat 6-well tissue culture plates with poly-D-lysine (PDL, 10 µg/mL in PBS) at 4 °C overnight. Remove the PDL and rinse with 1.5 mL of PBS per well.
    3. Proceed with coating the plates with laminin (10 µg/mL in PBS) at 37 °C for 2 h. Rinse the plates with 1.5 mL of PBS per well.
    4. Prepare DRG neuron maintenance medium, comprised of neurobasal medium supplemented with B27 (2% v/v), L-glutamine (1% v/v), nerve growth factor (NGF, 20 ng/mL), and P/S (1% v/v).
    5. Prepare DRG neuron purification medium, comprised of neurobasal medium supplemented with B27 (2% v/v), L-glutamine (1%), NGF (20 ng/mL), P/S (1%), fluorodeoxyuridine (FDU, 10 µg/mL), and uridine (10 µg/mL).
    6. Sacrifice pregnant rats at gestational day 14-15 by pentobarbital overdose (240 mg/kg bodyweight, intraperitoneal).
    7. Place the sacrificed animals in supine position. Clean their abdomen thoroughly with 70% ethanol.
    8. Cut the lower abdominal wall of the animal longitudinally using fine dissecting scissors and forceps. Identify and remove the uterus using dissection scissors. Cut the uterine wall to expose and extract the embryos. Transfer the embryos to a sterile, 10 cm culture dish filled with PBS. Place the culture dish on ice.
    9. Transfer the embryo intended for dissection to a sterile, 10 cm culture dish filled with PBS (room temperature) and position it beneath a dissection microscope. Have the embryo in prone position.
        NOTE: The whitish spinal cord and the attached DRGs can be seen over the dorsal aspect of the embryo, through its translucent skin.
    10. Insert microdissecting forceps along either side of the spinal cord and use blunt dissection to begin separating the spinal cord from the surrounding soft tissue. Cut the spinal cord free from the animal using microdissecting forceps along the neck opening and tail stub. Perform further blunt dissection over the ventral aspect of the cord to free it from surrounding soft tissue.
    11. Use microdissecting forceps to remove residual soft tissue over the dorsal aspect of the freed spinal cord.
        NOTE: At this stage, only the spinal cord, nerve roots, and attached DRG should remain.
    12. Detach individual DRGs from their connecting nerve roots using microdissecting forceps. Use a pipette pen attached to a 1 mL tip to transfer the DRGs to a 1.5 mL, sterile centrifuge tube containing PBS.
        NOTE: For each 1.5 mL tube, a maximum of 100 DRGs can be accommodated.
    13. Centrifuge the DRGs at 250 x g for 5 min and resuspend them in recombinant enzymatic cell dissociation reagent (200 µL per tube). Incubate (37 °C, 5% CO_2) for 10 min. Centrifuge the DRGs at 250 x g for 5 min, remove the supernatant, and resuspend in DRG neuron maintenance medium. Dissociate the pellet by gentle trituration using a 200 µL pipette tip. Quantify the cells within the pellet using a hemocytometer after an appropriate dilution.
    14. Seed the cells at a density of 5,000 cells/cm^{2} into PDL/laminin-coated 6-well plates in 1.5 mL of DRG neuron maintenance medium per well. After two days of culture, remove the DRG neuron maintenance medium, rinse with PBS, and replace with DRG neuron purification medium.
        NOTE: For each purification cycle, treat the DRG cultures with purification medium for 2 days, followed by 1 day of incubation in maintenance medium. After 3-4 purification cycles, the removal of all endogenous glia is expected[7]. This should take approximately 14 days. Purified cultures test positive for the neuronal marker TUJ1 and are absent for S100β expression (**Figure 5**).

2. **Generation of Schwann cell-like cells**
    1. Prepare glial induction medium comprised of αMEM supplemented with β-Heregulin (100 ng/mL), bFGF (10 ng/ml), platelet-derived growth factor (PDGF-AA, 5 ng/mL), FBS (10%), and P/S (1% v/v).
    2. Plate the neurospheres prepared in section 4 in PDL/laminin-coated 6-well plates at a density of 5-10 spheres per cm[2] in 1.5 mL of glial induction medium per well. Replace glial induction medium every 2 days after rinsing the cells with PBS.
        NOTE: Cells from seeded neurospheres are seen to migrate outwards by day 2. By day 7, migratory cells have a tapered appearance and should demonstrate immunopositivity for the Schwann cell markers p75 neurotrophin receptor (p75) and S100β[7]. These cells are referred to as SCLCs.

3. **Coculture of SCLCs with DRG neurons**

1. Prepare coculture medium comprised of DRG neuron maintenance medium (step 5.1.4) and glial induction medium (step 5.2.1) at a 1:1 volume-to-volume ratio.
2. Prepare Schwann cell maintenance medium comprised of DMEM/F12 supplemented with FBS (5%), β-Heregulin (10 ng/mL), and P/S (1% v/v).
3. Remove the culture medium from day 7 SCLCs, rinse them with PBS, and incubate them with 0.5 mL/well of recombinant enzymatic cell dissociation reagent at 37 °C for 5 min. Resuspend the SCLCs in coculture medium.
4. Quantify the cells using a hemocytometer after an appropriate dilution.
5. Seed the SCLCs onto purified DRG neuron cultures at a density of 1,000 cells/cm^2. Maintain the cocultures for 14 days, with medium replacement every 2 days.
   NOTE: During coculture, SCLCs acquired the spindle-like morphology that is typical of mature Schwann cells (**Figure 6**). These cells persist in their phenotype after the withdrawal of growth factors and are able to myelinate axons *in vitro* and *in vivo*[7,10]. The positivity of Schwann cell markers (*i.e.,* p75 and S100β) should be monitored by immunofluorescence.
6. Upon completion of coculture, passage fate-committed Schwann cells, as described in step 1.2.2. Use 0.5 mL of dissociation reagent per well. Quantify the cells using a hemocytometer after an appropriate dilution.
7. Resuspend fate-committed Schwann cells in Schwann cell maintenance medium at a density of 10,000 cells/cm^2. Seed cells into PDL/laminin-coated 6-well plates for immunofluorescence.
   NOTE: Cocultures inevitably contain MSCs that that have adopted a fibroblast cell fate[7]. These cells are passaged together with fate-committed Schwann cells upon the completion of the coculture. Schwann cells lying on top of this fibroblast substratum can be easily detached and further expanded following rinsing with "cold jets" of PBS (4 °C), as has been described elsewhere[13]. Fate-committed Schwann cells can be expanded in maintenance medium for 1 month.

Representative Results

An overview of the key stages in our protocol is illustrated in **Figure 1**. In summary, rat and human MSCs are selected for by adherence to tissue culture plastic. Expanded MSCs are preconditioned with hypoxia and are then subject to neurosphere-forming conditions. Neurospheres are plated and allowed to differentiate into SCLCs. SCLCs are cocultured with purified DRG neurons to generate fate-committed Schwann cells.

The morphology of cultured rat and human MSCs is illustrated in **Figure 2**. Their healthy tapered morphology is shown in contrast to the quadrangular appearance of MSCs maintained for high passage numbers, which have lost their multipotency. Expanded rat and human colonies should demonstrate the expression of MSC markers, an absence of hematopoietic stem cell markers, and the capacity for trilineage differentiation (**Figure 3**). Healthy MSCs from between passages 3 and 8 are subject to hypoxic preconditioning for 16 h and are thereafter passaged onto low-adherence culture plates with EGF/bFGF supplementation. Hypoxic preconditioning results in larger numbers of neurospheres, as well as larger average neurosphere sizes (**Figure 4**).

Neurospheres are plated onto PDL/laminin-coated culture plates and induced to become SCLCs by culture in glial induction medium containing β-Heregulin, bFGF, and PDGF-AA. SCLCs exhibit the characteristic tapered morphology of Schwann cells and the corresponding marker expression, yet they are phenotypically unstable and revert to a fibroblast phenotype upon the discontinuation of growth factors

Coculture with sensory neurons is a prerequisite to bring about a cell-intrinsic switch to fate commitment. The establishment of purified DRG networks is achieved by means of pulsed treatment with FDU and uridine to remove endogenous glia and should be confirmed by the absence of S100β immunopositivity (**Figure 5**). On day 7, SCLCs are passaged and cocultured with purified DRG neurons for 14 days (**Figure 6**). Upon the completion of coculture, mature, fate-committed Schwann cells should emerge.
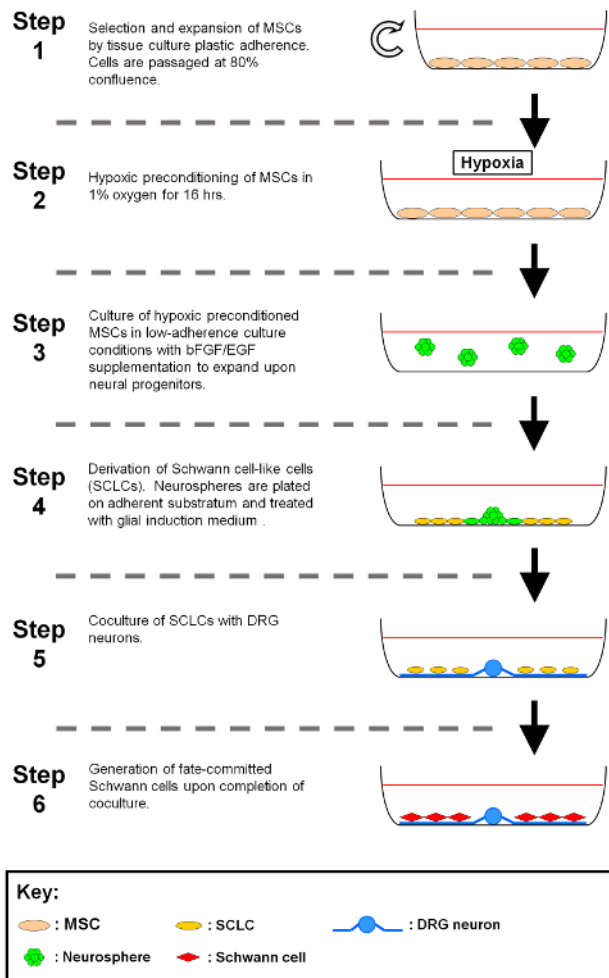
Figure 1: Overview of the protocol. Bone marrow is obtained from either rat femurs or human iliac crest aspirates. MSCs from within the bone marrow can attach and expand upon tissue culture plastic. In order to enrich for MSCs with neural potential, cells are preconditioned in 1% O_2 for 16 h and are then passaged onto low-adherence culture plates with bFGF/EGF supplementation. This results in the formation of neurospheres, which are plated onto PDL/laminin-coated tissue culture plastic and cultured in glial induction medium to generate SCLCs. SCLCs are passaged and cocultured with purified DRG neurons for 2 weeks in order to direct them to maturity. Please click here to view a larger version of this figure.
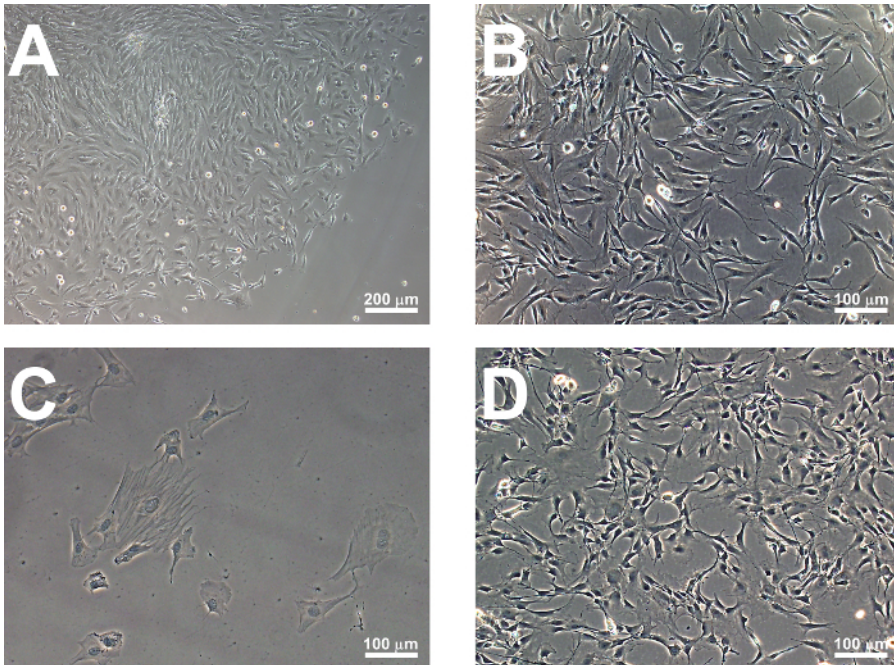
Figure 2: Establishment of MSC colonies. Sizeable MSC colonies should be visible 6-7 days after the plating of bone marrow cells onto tissue culture plastic. A representative image of a rat MSC colony is shown (**A**), while human colonies demonstrate a similar appearance. Colonies can be passaged on day 10. Seen at higher magnification, both rat (**B**) and human (**D**) MSCs exhibit a characteristic fibroblast-like morphology after passaging. MSCs that are maintained for high passage numbers acquire a flattened, quadrangular morphology (**C**) and should be discarded. Please click here to view a larger version of this figure.
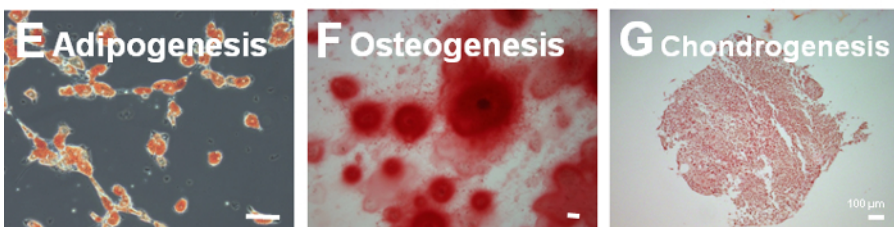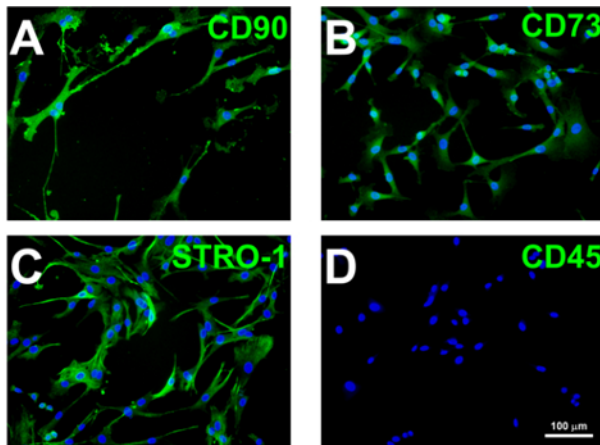

Figure 3: Characterization of MSCs. (**A**-**D**) Representative images of human MSCs. MSCs can be characterized by the expression of appropriate markers, such as CD90 (**A**), CD73 (**B**), and Stro-1 (**C**), and by the absence of hematopoietic stem cell markers, such as CD45 (**D**). (**E**-**G**) Rat MSCs isolated and expanded as described in the protocol demonstrate multipotency in their ability to form adipocytes (**E**; fat deposits stained with Sudan Red), osteoblasts (**F**; pericellular matrix stained with Alizarin Red), and chondrocytes (**G**; proteoglycans stained with Safranin-O) under appropriate culture conditions. Scale bars = 100 µm. Please click here to view a larger version of this figure.

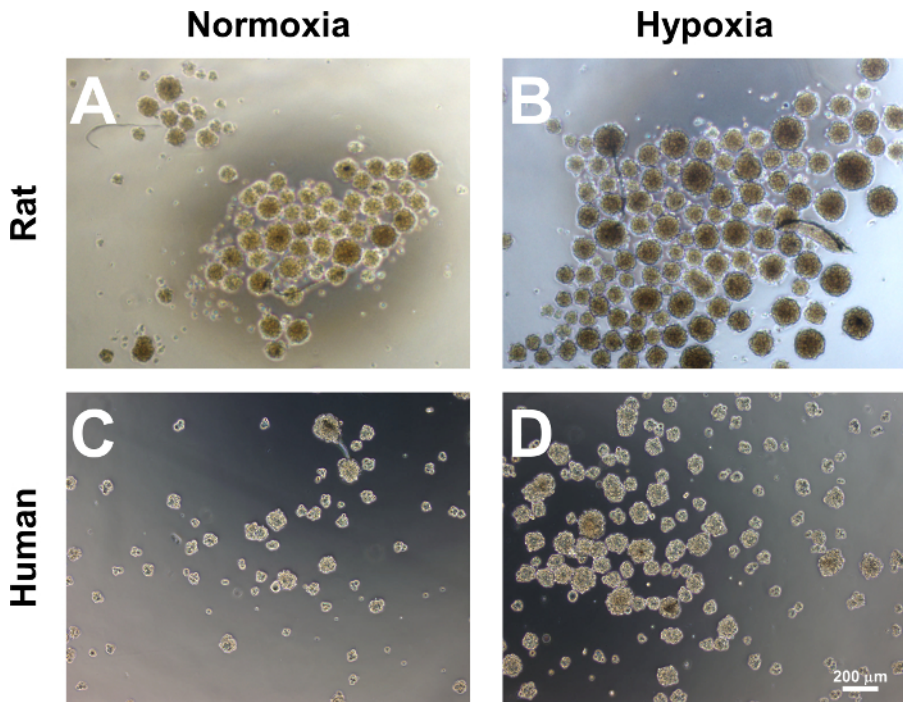**Normoxia**   **Hypoxia**

Figure 4: Enrichment of neural progenitors from MSCs. Both rat (**A**) and human (**C**) MSCs form neurospheres when cultured upon low-adherence tissue culture plastic in medium supplemented with EGF/bFGF. The numbers and average diameter of rat (**B**) and human (**D**) spheres is enhanced via the hypoxic preconditioning of MSCs (16 h, 1% O_2) prior to sphere induction. Scale bars = 200 μm. Please click here to view a larger version of this figure.
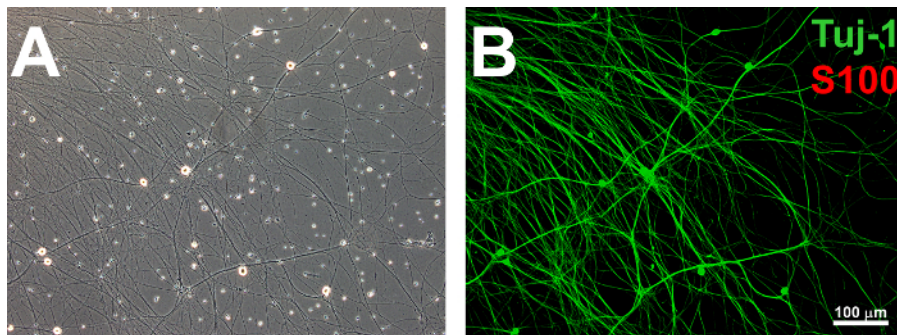

Figure 5: Establishing purified rat DRG networks. Purified DRG networks are established after pulsed treatment with the antimitotic agents FDU and uridine (A). Neurite networks that are devoid of S100β-expressing endogenous glia (B) are ready for coculture with SCLCs. Scale bars = 100 μm. Please click here to view a larger version of this figure.
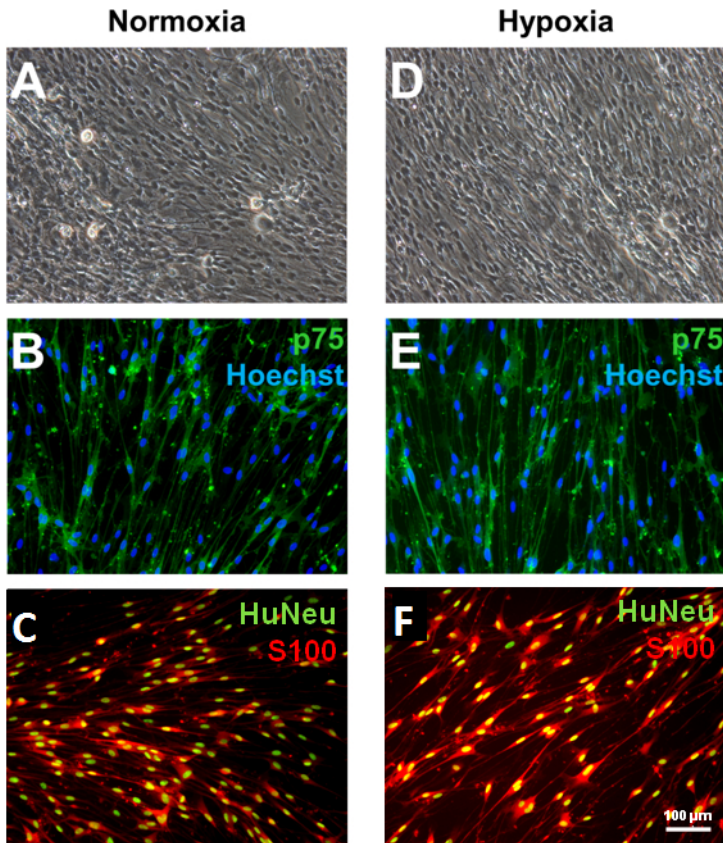
Figure 6: Generation of bone marrow-derived Schwann cells via coculture with DRG neurons. Upon completing 2 weeks of coculture with purified DRG neurons and human SCLCs, spindle-shaped, fate-committed Schwann cells emerge from both normoxia- and hypoxia-treated groups (**A** and **D**). These cells express the Schwann cell markers p75 (B and E) and S100β (C and F). The expression of human nuclei antigen (HuNeu) demonstrates that the S100β-positive cells were not contaminating glial cells originating from rat DRGs. Scale bars = 100 μm.

Discussion

It is essential to preserve the "stemness" of MSCs prior to the enrichment of neural progenitors via hypoxic preconditioning and neurosphere culture. From our experience, multipotent MSCs can be reliably identified by their elongated fibroblast-like morphology. In contrast, MSCs that have adopted a more flattened, quadrangular morphology, with prominent cytoskeletal stress fibers, do not readily adopt neural cell fates and should be discarded. In general, we do not utilize MSCs with passage numbers greater than eight. To preserve their stemness, it is critical to promptly passage MSCs before they reach 100% confluence. Conversely, maintaining MSCs at a too-low confluence is undesirable. From our experience, seeding MSCs at a density of 40,000 cells/cm^2, or simply passaging cells that are 80% confluent at a 1:2 ratio, allows for the best results.

The proper establishment and maintenance of the DRG network is a critical determinant of coculture success. The time required for DRG harvest should be kept to a minimum. Individual ganglia should be handled in an atraumatic manner, particularly during detachment from the spinal cord, when it is best to handle the nerve roots only. In general, we aim for a period of less than 2 h between the time of animal sacrifice and the enzymatic digestion of harvested DRGs, as a prolonged harvest results in tissue maceration and the loss of cell viability. The detachment of DRG neurons from the substratum during culture is often encountered. To prevent this from occurring, the coating should be freshly prepared and performed near the time of tissue harvest. In general, large, undigested DRG clusters detach more often and do not yield coculture success. The duration of enzymatic digestion and the amount of trituration can be adjusted, with the aim of achieving a network with an appearance resembling **Figure 5**.

While our coculture platform consistently induces fate commitment, only 20-30% of cultures performed in parallel yield fate-committed Schwann cells[7,13]. We therefore prepare enough DRGs and SCLCs to concomitantly perform cocultures in three to four 6-well culture plates. We hypothesize that a combination of factors related to the underlying DRG network, including their embryonic age, cell viability, density, and topography, affect coculture success. These underlying variables need to be further investigated and standardized. Apart from limitations in the coculture yield, a means of superseding the requirement for rat-derived DRG neurons and animal products should be sought. Furthermore, the duration of the protocol should be shortened. As an abbreviated modification of our protocol, we have had success in deriving mature Schwann cells from day 10 neurospheres seeded directly onto purified DRG neurons, without the prior generation of SCLCs[7,10].

Neurospheres enriched via our method serve as a robust source of cells of neuronal and glial lineages. Our platform for directing precursor cells to fate commitment has the advantage of avoiding genetic manipulation, with its inherent risks, and the resultant cells are of relevance for cell transplantation, disease modeling, and the study of glial differentiation.

Disclosures

All authors of this manuscript have no disclosures to declare.

Acknowledgements

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