

Application Note

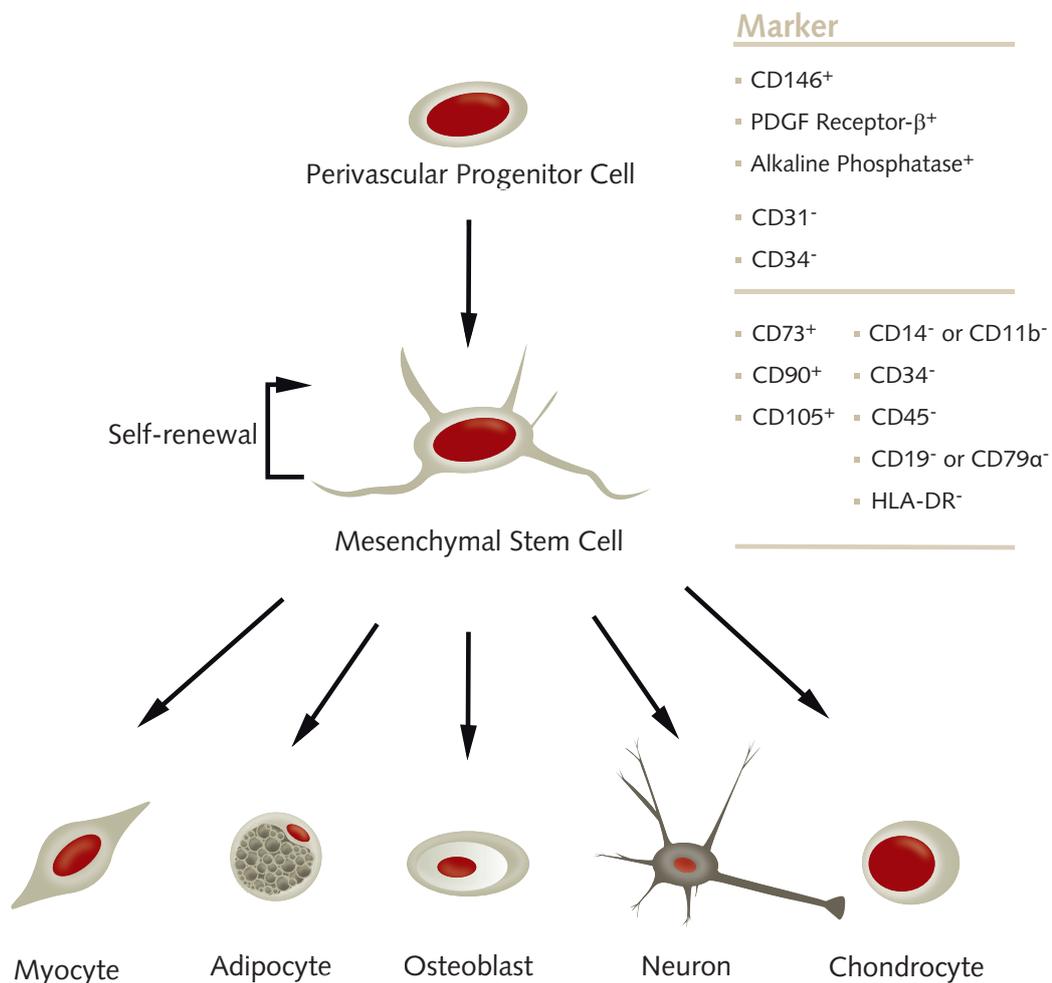
Background

Mesenchymal stem cells (MSC) are fibroblastoid multipotent adult stem cells with a high capacity for self-renewal. So far, these cells have been isolated from several human tissues, including bone marrow, adipose tissue, umbilical cord matrix, tendon, lung, and the periosteum [1]. Recently it has been shown that MSC originate from the perivascular niche, a tight network present throughout the vasculature of the whole body. These perivascular cells lack endothelial and hematopoietic markers, e.g. CD31, CD34 and CD45, but express CD146, PDGF-R beta, and alkaline phosphatase [2].

Characterization

According to the position paper published by the International Society for Cellular Therapy (ISCT), MSC express the surface markers CD73, CD90 and CD105 and stain negative for CD14 or CD11b, CD34, CD45, CD79 α or CD19, and HLA-DR [3]. In addition to surface marker analysis, the most common and reliable way to identify a population of MSC is to verify their multipotency. MSC can differentiate into adipocytes, osteoblasts, myocytes, and chondrocytes *in vivo* and *in vitro* [1,4]. Trans-differentiation of MSC into cells of non-mesenchymal origin, such as hepatocytes, neurons

and pancreatic islet cells, has also been observed *in vitro* when specific culture conditions and stimuli are applied [1]. The directed differentiation of MSC is carried out *in vitro* using appropriate differentiation media, such as the ready-to-use PromoCell MSC Differentiation Media (see below for differentiation protocol). Terminally differentiated cells are histochemically stained to determine their respective identities (see below for staining protocol).



Use aseptic techniques and a laminar flow bench.

Neurogenic Induction

1. Coat the culture vessel

Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin (C-43060/C-43050) according to the instruction manual.

2. Seed the Mesenchymal Stem Cells

Plate 4×10^3 cells/cm² on the fibronectin-coated plate using MSC Growth Medium 2 (C-28009). Work in duplicate.

3. Let the Mesenchymal Stem Cells grow

Culture the cells to 60–80% confluency. Change the medium every 48 hours.

4. Induce the Mesenchymal Stem Cells

Induce one of the duplicate samples with MSC Neurogenic Differentiation Medium (C-28015). Use MSC Growth Medium 2 (C-28009) for the remaining well as a negative control.

5. Differentiation of the Mesenchymal Stem Cells

Incubate for at least 3 days. Change the medium every 48 hours.

Note: Significant morphological changes in the cells can be observed as early as 1 day after induction (Fig. 1).

6. Harvest and characterize the cells

The MSC-derived neuronal cells are now ready to be used in your experiments. If required, the neuronal cells can be characterized further by proceeding with the following protocol, "Detection of neuronal markers".

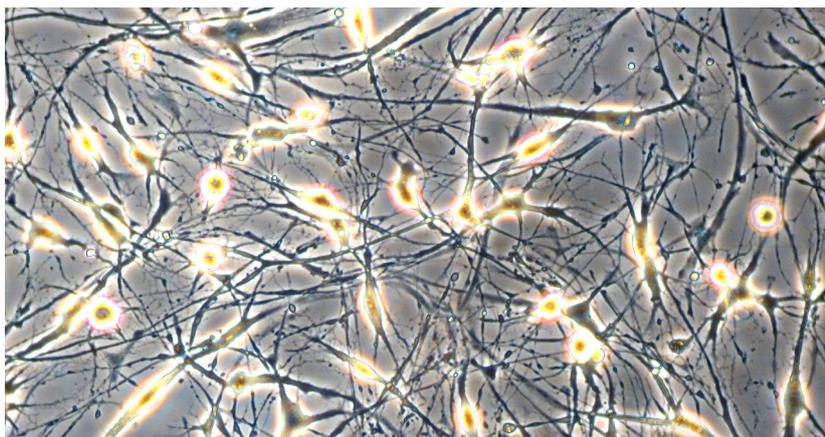


Fig. 1: Neuron-like cells generated from hMSC-BM (human MSC derived from bone marrow) in PromoCell MSC Neurogenic Differentiation Medium (C-28015). Note the formation of axon- and dendrite-like cellular structures.

Please follow the recommended safety precautions for the chemicals used in this procedure!

Detection of Neuronal Markers

Differentiation of MSC into cells of neuronal lineage is accompanied by striking changes in cell morphology, namely the formation of dendrites and axons (see Fig. 1). Thus, the neuronal induction process can be monitored easily.

Morphological changes, however, may not characterize putative neuronal cells sufficiently. One histochemical technique for the detection of neuronal cells is the specific staining of neuronal Nissl bodies. These characteristic granular structures are composed of RNA-rich rough endoplasmic reticulum (rER) and are unique to the somata of neurons.

Nissl body staining protocol

1. Prepare reagents and buffers

Use Saccomanno Fixation Solution (Morphisto, #13881.00250). Prepare the Nissl staining solution (0.5% cresyl violet) as follows: make 0.6 ml of glacial acetic acid up to 100 ml with distilled water. Add 0.5 g of cresyl violet acetate and stir for 20 min. Pass through a 0.22 μm filter. Store in the dark in a tightly closed container at room temperature and use within 6 months.

2. Wash the cells

Remove the cells from the incubator, aspirate the medium and gently wash the cell monolayer twice with phosphate-buffered saline (PBS) w/o $\text{Ca}^{++}/\text{Mg}^{++}$.

3. Fix the cells

Aspirate the PBS and fix the cells with Saccomanno Fixation Solution for at least 30 min at room temperature. Use enough fixative to cover the cell monolayer completely.

4. Wash the cells

Aspirate the fixation solution and gently wash the cell monolayer twice with PBS.

5. Stain the cells

Immediately before use, pass the required amount of Nissl staining solution through a 0.22 μm syringe filter equipped with a PES-membrane. Remove the PBS from the cells and add the staining solution. Use enough staining solution to cover the cell monolayer completely. Incubate at room temperature for 30 min.

6. Wash the cells

Aspirate the staining solution. Gently wash the cell monolayer three times with PBS.

Detection of Neuronal Markers

Please follow the recommended safety precautions for the chemicals used in this procedure!

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7. Detect results

Cover the stained cells with PBS and evaluate the samples promptly as the dye may bleed upon prolonged storage. Use a microscope at low magnification (40–50x) in bright field mode.

Results: nuclei stain a light blue/violet color, Nissl bodies appear dark black-violet with the background remaining almost colorless (see Fig. 2).

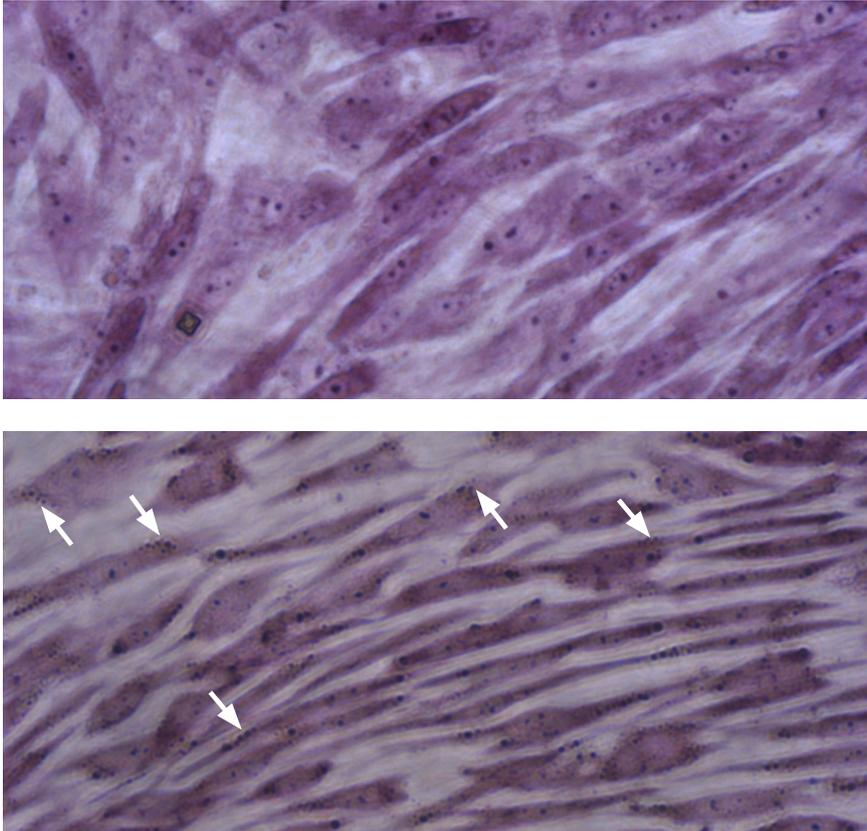


Fig. 2: Nissl body staining of hMSC-BM derived neuron-like cells. Cells were cultured for 3 days in PromoCell MSC Growth Medium 2 (C-28009) for the negative control (top panel) or MSC Neurogenic Differentiation Medium (C-28015) for the differentiation sample (bottom panel). In contrast to the negative control, the neuronal cells differentiated from MSC show extensive somata-associated accumulations of Nissl bodies stained dark black-violet (white arrows).

Detection of Neuronal Markers



References

- [1] da Silva Meirelles L, Caplan AI, Nardi NB., Stem Cells 2008; 26(9):2287–99.
[2] Crisan M, Yap S, Casteilla L, et al., Cell Stem Cell 2008; 3(3):301–13.
[3] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, et al., Cytother 2006; 8(4):315–7.
[4] Caplan AI., Cell Stem Cell 2008; 3(3):229–30.

Related Products

| Product | Size | Catalog Number |
|---|--|--------------------|
| Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM) | 500,000 cryopreserved cells 500,000 proliferating cells | C-12974 C-12975 |
| Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC) | 500,000 cryopreserved cells 500,000 proliferating cells | C-12971 C-12972 |
| Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT) | 500,000 cryopreserved cells 500,000 proliferating cells | C-12977 C-12978 |
| Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use) | 500 ml | C-28009 |
| Mesenchymal Stem Cell Growth Medium DXF (Ready-to-use) | 500 ml | C-28019 |
| Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (Ready-to-use) | 100 ml | C-28016 |
| Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use) | 100 ml | C-28012 |
| Mesenchymal Stem Cell Chondrogenic Differentiation Medium w/o Inducers (Ready-to-use) | 100 ml | C-28014 |
| Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use) | 100 ml | C-28013 |
| Mesenchymal Stem Cell Neurogenic Differentiation Medium (Ready-to-use) | 100 ml | C-28015 |
| Accutase-Solution, primary human cell culture tested | 100 ml | C-41310 |
| Cell Dissociation Solution ACF | 100 ml | C-41320 |
| Dulbecco's PBS, w/o Ca ⁺⁺ / Mg ⁺⁺ | 500 ml | C-40232 |
| Fibronectin Solution, human (1 mg/ml) | 5 ml | C-43060 |
| Fibronectin Solution, bovine (1 mg/ml) | 5 ml | C-43050 |
| hMSC-BM Pellet | 1 million cells per pellet | C-14090 |
| hMSC-UC Pellet | 1 million cells per pellet | C-14091 |
| hMSC-AT Pellet | 1 million cells per pellet | C-14092 |

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