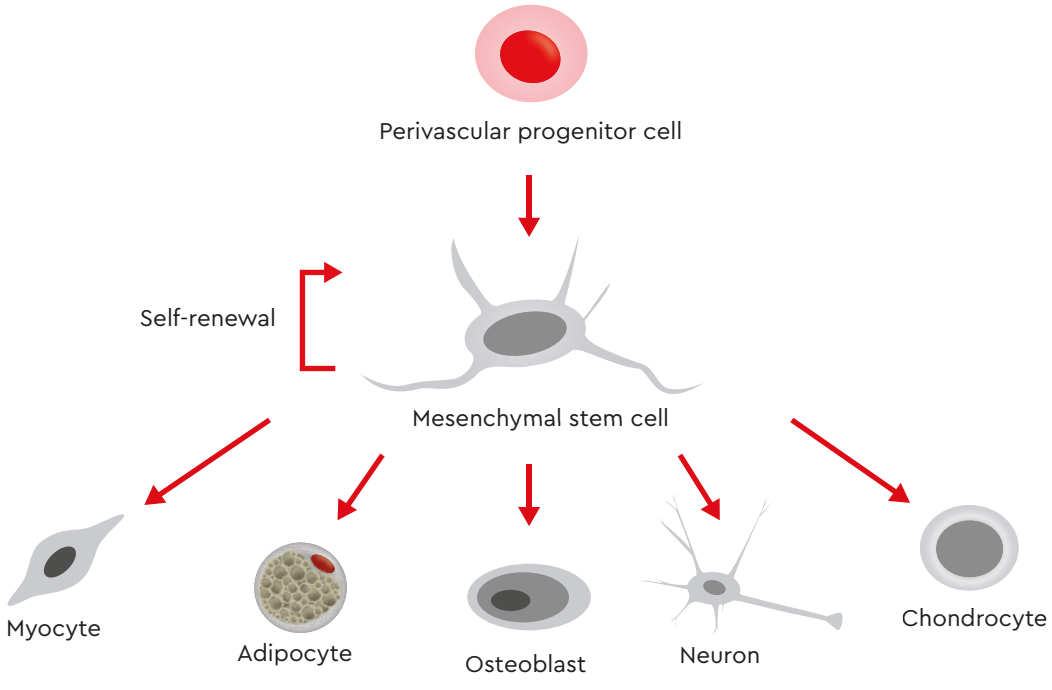
Neurogenic differentiation and analysis of MSC

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].

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, CD79α 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]. Transdifferentiation 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).



Marker

Perivascular progenitor cell

- CD146$^+$
- PDGF Receptor -β$^+$
- Alkaline Phosphatase$^+$
- CD31$^-$
- CD34-

Mesenchymal stem cell

- CD73$^+$
- CD90$^+$
- CD105$^+$
- CD14$^-$ or CD11b$^-$
- CD34$^-$
- CD45$^-$
- CD19$^-$ or CD79α$^-$
- HLA-DR-

Protocol for neurogenic differentiation and analysis of MSC

I. Neurogenic induction

Materials

- Human Mesenchymal Stem Cells from bone marrow (hMSC-BM, C-12974)
- Mesenchymal Stem Cell Growth Medium 2 (C-28009)
- Mesenchymal Stem Cell Neurogenic Differentiation Medium (C-28015)
- Fibronectin, human or bovine
- Tissue culture treated cell culture vessels

Use aseptic techniques and a laminar flow bench.

1

Coat the culture vessel

Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin 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 for the remaining well as a negative control.

5

Differentiation of the mesenchymal stem cells

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

Note: Significant morphological changes in the cells can be observed as early as one 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".

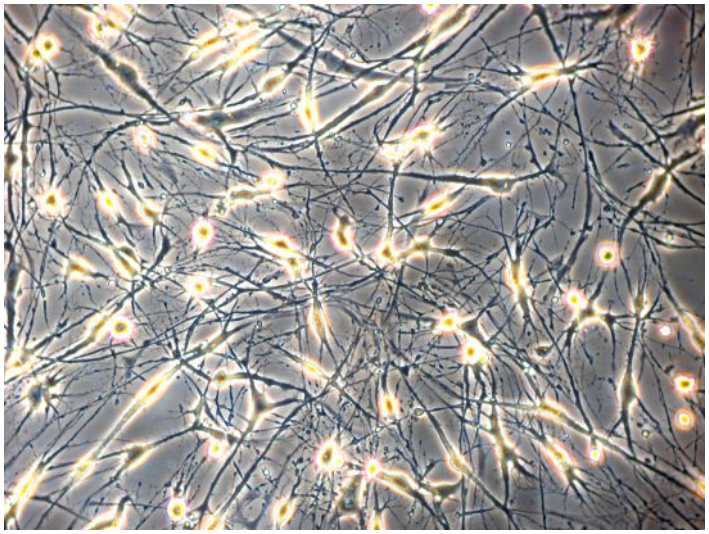


Fig. 1: Neuron-like cells generated from hMSC-BM (human MSC derived from bone marrow) in our MSC Neurogenic Differentiation Medium. *Note the formation of axon- and dendrite-like cellular structures.*

II. Detection of neuronal markers: Nissl body staining protocol

Materials

- Saccomanno Fixation Solution (Morphisto, Cat. No. 13881.00250)
- Nissl staining solution
- Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++} (C-40232)
- Syringe filter, 0.22 µm

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

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.

1

Prepare reagents and buffers

Use Saccomanno Fixation Solution to prepare the Nissl staining solution (0.5% cresyl violet) as follows: fill 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 minutes. 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 Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++}.

3

Fix the cells

Aspirate the PBS and fix the cells with Saccomanno Fixation Solution for at least 30 minutes 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 minutes.

6

Wash the cells

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

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 (40–50 x) 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).

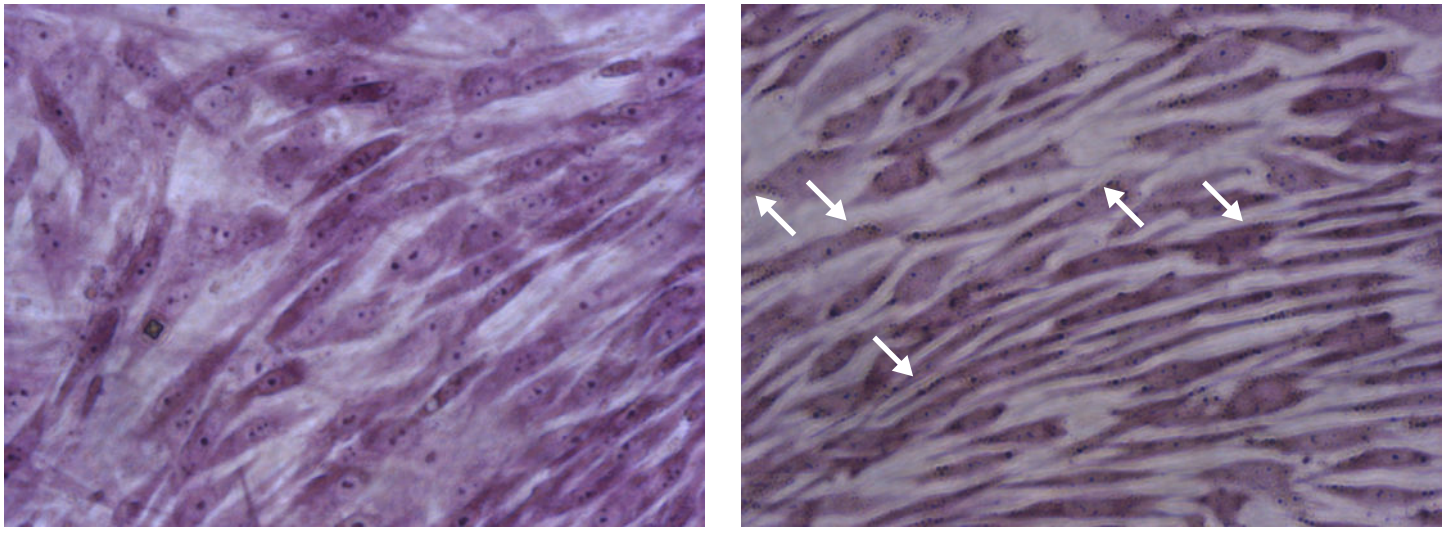


Fig. 2: Nissl body staining of hMSC-BM derived neuron-like cells. *Cells were cultured for 3 days in our MSC Growth Medium 2 (C-28009) for the negative control (left panel) or MSC Neurogenic Differentiation Medium (C-28015) for the differentiation sample (right panel). In contrast to the negative control,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

Related Products

Media	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 XF (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 without 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
Dulbecco's PBS, without Ca^{++}/ Mg^{++}	500 ml	C-40232
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

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):301–3.
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.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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