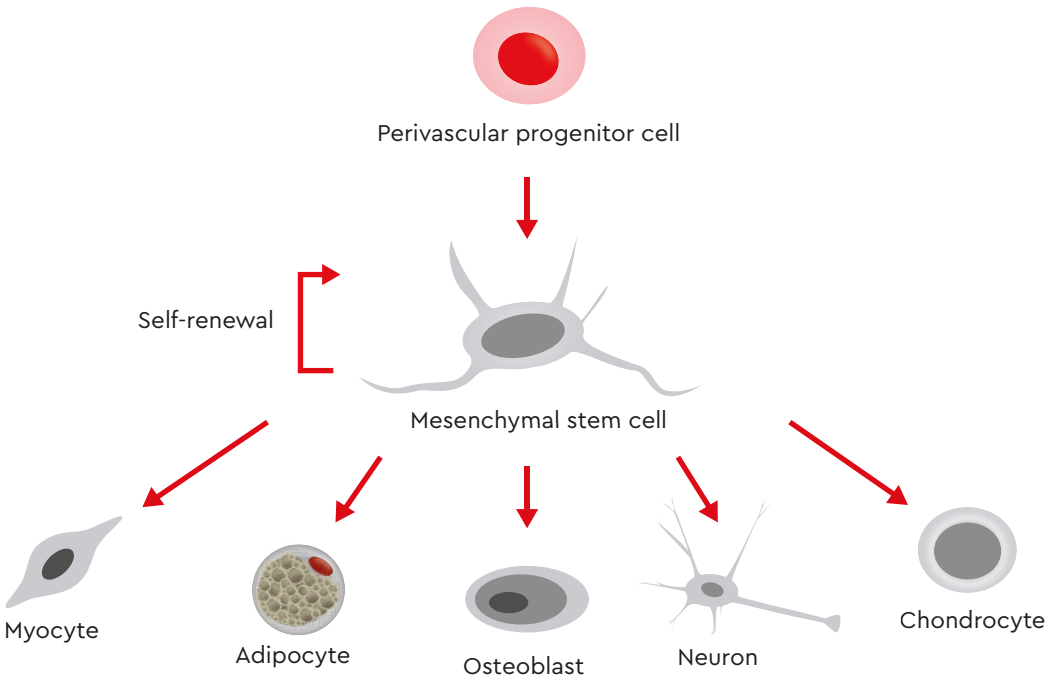
Adipogenic differentiation and analysis of MSC

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 our ready-to-use 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 adipogenic differentiation and analysis of MSC

I. Differentiation protocol

Materials

- Human Mesenchymal Stem Cells from bone marrow (hMSC-BM, C-12974)
- Mesenchymal Stem Cell Growth Medium 2 (C-28009)
- Mesenchymal Stem Cell Adipogenic Differentiation Medium (C-28016)
- 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 human or bovine fibronectin according to the instruction manual.

2

Seed mesenchymal stem cells

In a fibronectin-coated 6-well tissue culture plate, plate 1×10^5 MSC per well using MSC Growth Medium 2 (C-28009). Work in duplicate.

3

Let the mesenchymal stem cells grow

Allow the cells to reach 80–90% confluency. This will take 24–48 hours.

4

Induce the mesenchymal stem cells

Induce one of the duplicate samples with MSC Adipogenic Differentiation Medium 2 (C-28016). Use MSC Growth Medium 2 for the remaining well as a negative control.

5

Differentiation of the induced mesenchymal stem cells

Incubate for 12–14 days. Change the medium every third day taking care not to disturb the cell monolayer.

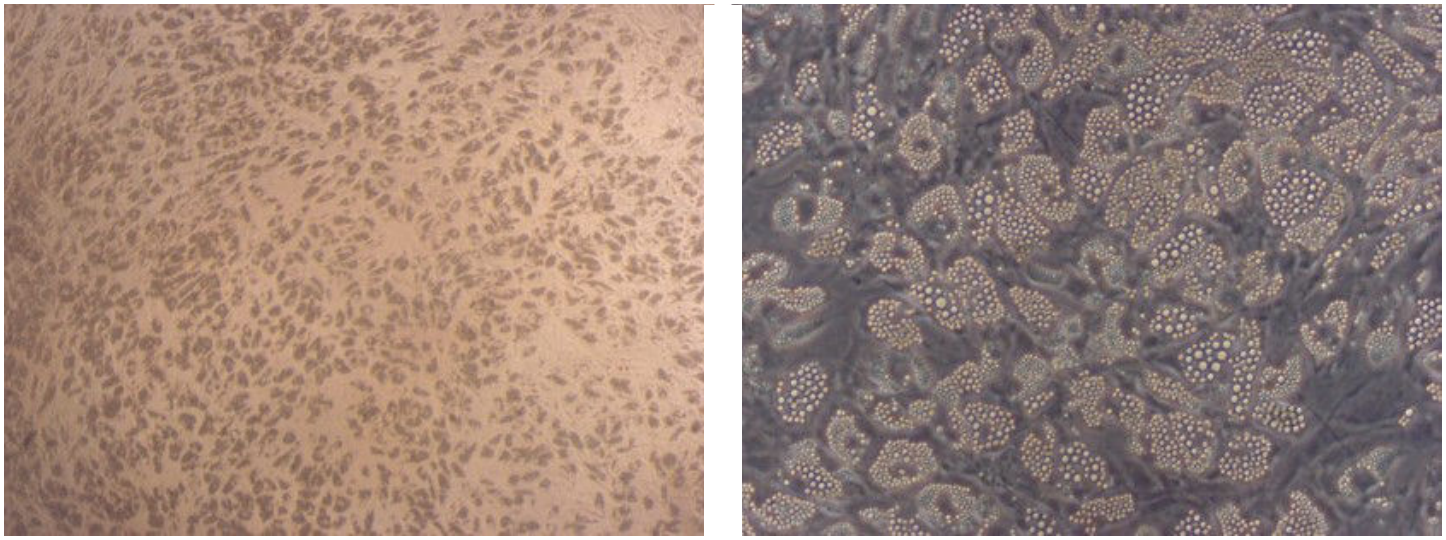


Fig. 1: Lipid vesicle accumulation in adipocytes differentiated from hMSC-BM (human MSC derived from bone marrow) using our MSC Adipogenic Differentiation Medium 2 (C-28016). *The differentiated culture exhibits extensive intracellular lipid vacuole formation typical of mature adipocytes (left: 40x magnification; right: 100x magnification).*

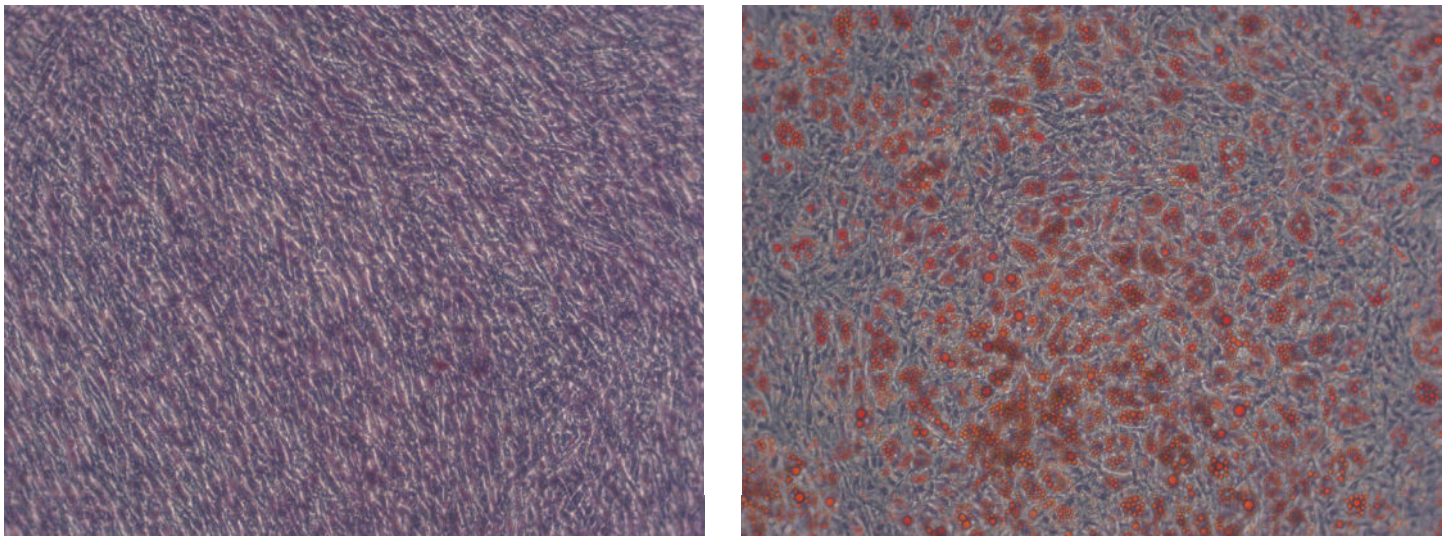


Fig. 2: Sudan III staining of intracellular lipids in hMSC-BM-derived mature adipocytes. *The cells were cultured for 12 days in our MSC Growth Medium 2 (C-28009) as a negative control (left) or the MSC Adipogenic Differentiation Medium 2 (C-28016) for the differentiation sample (right). In contrast to the negative control, the mature adipocytes differentiated from MSC exhibit intracellular lipid vesicles (bright red staining).*

II. Adipocyte detection

Materials

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

Important: Do not let the cells dry for longer than 30 seconds throughout the entire staining procedure

In mature adipocytes intracellular lipid vesicles are typically observed in large numbers (Fig. 1). These can be highlighted using a lipophilic dye, such as Sudan III, which stains lipid accumulations bright red (Fig. 2).

1

Prepare solutions and buffers

Use Saccomanno Fixation Solution (Morphisto, Cat. No. 13881.00250) and Sudan III Solution (Morphisto #10396.00500). Prepare a 60% isopropanol solution with distilled water.

2

Wash the cells

Remove the cells from the incubator and carefully aspirate the medium. Gently wash the cells with Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++} (C-40232).

Note: Do not disrupt the cell monolayer!

3

Fixation of the cells

Carefully aspirate the PBS and add enough Saccomanno Fixation Solution to cover the cell monolayer. Incubate at room temperature for at least 30 min.

4

Dilute the staining solution

During fixation, dilute 10 ml Sudan III Solution with 1.5 ml distilled water and pass through a syringe filter. Use within 30 minutes.

5

Wash the cells

Carefully aspirate the fixation buffer and wash the cell monolayer with distilled water. Gently aspirate the water and add enough 60% isopropanol to cover the cell monolayer. Incubate at room temperature for 5 minutes.

6

Add the staining solution

Carefully aspirate the 60% isopropanol and add enough diluted Sudan III staining solution to cover the cell monolayer. Incubate at room temperature for 10–15 minutes.

7

Wash the cells

Carefully aspirate the staining solution and wash the cell monolayer several times with distilled water until the water is clear. Blot the vessel containing the stained cells upside down on a paper towel to remove as much water as possible.

8

Analyze the cells

Cover with PBS and analyze the stained samples promptly as the dye tends to fade upon prolonged light exposure. Intracellular lipid vesicles in mature adipocytes will be stained bright red (see Fig. 2).

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):301–13.
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.

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