Adipogenic differentiation of mesenchymal stem cells: A complete protocol with Oil Red O staining

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Application note

Adipogenic differentiation of mesenchymal stem cells (MSCs) into mature adipocytes is a key indicator of multipotency and a valuable tool in metabolic research and regenerative medicine. In this application note, we provide a step-by-step protocol for adipogenic differentiation of human mesenchymal stem cells (hMSCs).

Background

Mesenchymal stem cells (MSCs) are fibroblastoid multipotent adult stem cells with a high self-renewal capacity. Researchers can isolate MSCs from several human tissues, including bone marrow, adipose tissue, umbilical cord matrix, tendon, lung, and the periosteum.[1] Recent studies show that MSCs originate from the perivascular niche, a tightly organized cellular network that spans the vasculature throughout the body. These perivascular cells lack endothelial and hematopoietic markers (e.g., CD31, CD34, and CD45) but express CD146, PDGF-Rβ, and alkaline phosphatase.[2]

The International Society for Cellular Therapy (ISCT) has defined minimal criteria for MSC identification[3]:

- MSCs express CD73, CD90, and CD105
- They lack expression of CD14 or CD11b, CD34, CD45, CD79α or CD19, and HLA-DR

In addition to surface marker analysis, confirming multipotency is the most reliable way to identify MSCs. MSCs can differentiate into the following cell types *in vivo* and *in vitro*[1,4]:

- Adipocytes
- Neurons
- Osteoblasts
- Myocytes
- Chondrocytes

Trans-differentiation of MSCs into non-mesenchymal cell types, such as hepatocytes, neurons, and pancreatic islet cells, has also been observed *in vitro* under specific culture conditions and stimuli.[1]

Researchers can direct MSC differentiation *in vitro* using appropriate differentiation media, such as our ready-to-use PromoCell MSC Differentiation Media (see below for the differentiation protocol). Because MSCs have the potential to differentiate into multiple lineages, it is essential to verify the specific cell type they have developed into. In the case of adipogenic differentiation, differentiated adipocytes can be identified using Oil Red O staining. This lipophilic dye selectively labels intracellular lipid droplets in mature adipocytes with a bright orange-to-red coloration, providing clear visual confirmation of successful adipogenic differentiation (see below for staining protocol).

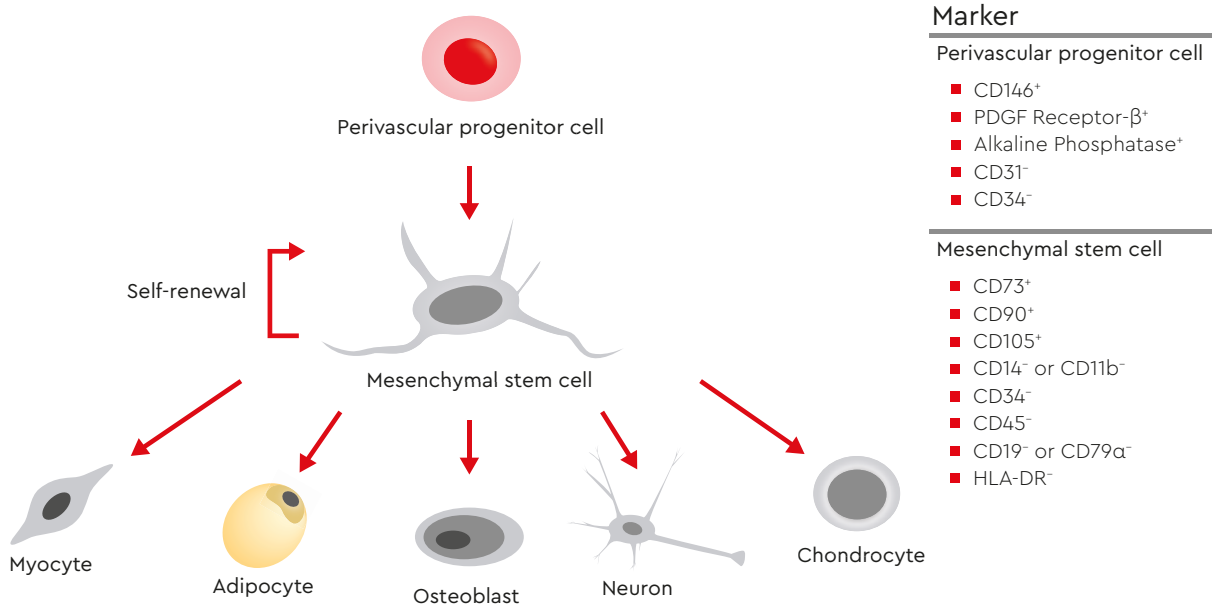


Fig. 1: Origin and multipotency of mesenchymal stem cells. *MSCs arise from perivascular progenitor cells and are characterized by their capacity for self-renewal and multipotent differentiation. Depending on the culture conditions applied, MSCs can give rise to adipocytes, osteoblasts, myocytes, neurons, and chondrocytes.*

Protocol

Protocol for adipogenic differentiation and analysis of MSCs

The protocol uses our ready-to-use Mesenchymal Stem Cell Adipogenic Differentiation Medium 2, followed by Oil Red O staining to visualize intracellular lipid accumulation in adipocytes.

I. Differentiation protocol

Materials

- Human Mesenchymal Stem Cells from bone marrow (hMSC-BM, C-12974)
- Mesenchymal Stem Cell Growth Medium 2 (C-28009 or C-28017)
- Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (C-28016)
- Recombinant human vitronectin (e.g., from ReliaTech GmbH or QKine)
- Tissue culture treated cell culture vessels (e.g., Falcon® Multiwell 6 well, catalog number 353046)
- Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++} (C-40232)

Note: Mesenchymal Stem Cell Growth Medium XF (C 28019 or C 28018) can also be used for MSC expansion in multipotency studies. For GMP work-flows, PromoExQ Mesenchymal Stem Cell Growth Medium 2 (EQ-C-28017) or PromoExQ MSC Growth Medium XF (EQ-C-28018) is recommended.

Use aseptic techniques and work within a laminar flow hood.

1

Coat the culture vessel

Prepare a recombinant human vitronectin stock solution of 100 µg/ml and store aliquots at -20°C. Avoid freeze and thaw cycles, and store thawed aliquots at 4–8°C (up to 3 months). Coat a 6-well tissue culture plate with 0.5 µg/cm^2 with recombinant human vitronectin. If you use a 100 µg/ml stock solution, dilute it 1:20 in PBS w/o Ca^{++}/Mg^{++} (Cat. No. C-40232), which will result in a 5 µg/ml solution. Transfer 1 ml of this diluted solution into the wells of a 6-well plate. Move the plate to ensure the entire area is covered with the coating solution. Incubate the coating solution at room temperature for 2 hours. Afterwards, wash the plate with 2 ml of PBS w/o Ca^{++}/Mg^{++}. Coated plates can be used directly or stored for up to 4 weeks at 4–8°C. It is important to keep the wells covered with PBS to prevent them from drying out.

2

Seed the mesenchymal stem cells

On the day of use, replace PBS with 2 ml of MSC Growth Medium 2 (C-28009 or C-28017) in each well of a precoated 6-well plate. Plate 1×10^5 MSCs per well (seeding density of ~10,000 cells/cm^2) using MSC Growth Medium 2. Work in duplicate.

3

Let the mesenchymal stem cells grow

Allow the cells to reach 80%–90% confluency. This will take 24–72 hours.

4

Induce 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 2–3 days (e.g., on Monday, Wednesday, and Friday), ensuring you do not disturb the cell monolayer.

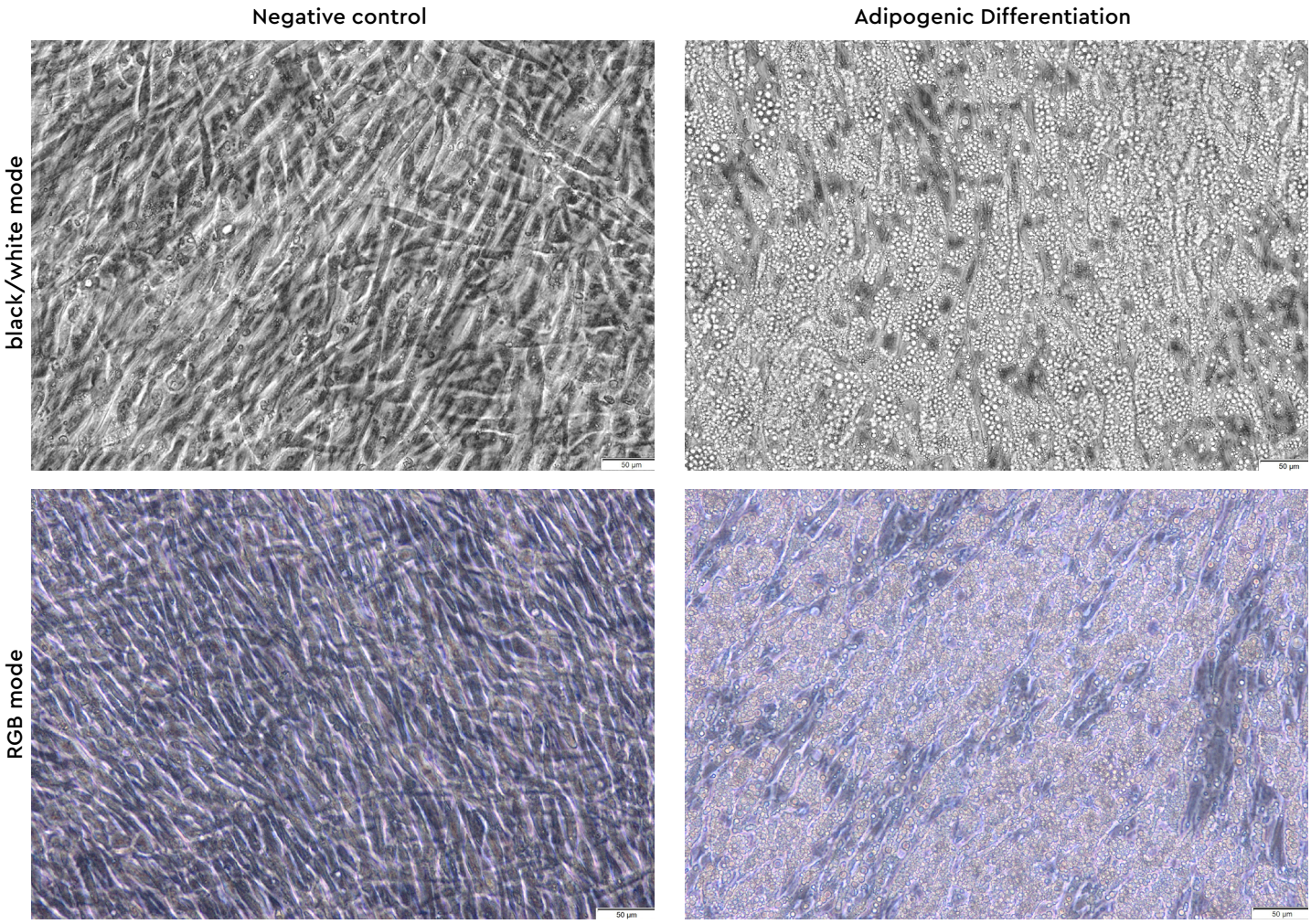


Fig. 2: Morphological changes and lipid droplet accumulation in adipocytes differentiated from hMSC-BM using Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (C-28016). *Representative brightfield microscopy images of unstained MSCs analyzed either in black/white mode (upper panel) or RGB mode (lower panel). The differentiated cells exhibit extensive formation of intracellular lipid droplets, typical of mature adipocytes (scale bar = 50 µm).*

II. Adipocyte detection

Materials

- Saccomanno Fixation Solution (Morphisto, Cat. No. 13881.00250)
- Oil Red O (Morphisto, Cat. No. 18575.00100)
- Harris Hematoxylin (Morphisto, Cat. No. 10222.00500)
- Isopropanol
- Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++} (C-40232)
- Syringe filter, 0.22 µm
- Folded filter, 100% cellulose

Important: Don´t let the cells dry for more than 30 seconds at any point during the staining process.

In mature adipocytes, intracellular lipid vesicles are typically observed in large numbers (Fig. 2). These can be highlighted using a lipophilic dye, such as Oil Red O, which stains lipid accumulations bright orange to red (Fig. 3).

1

Prepare solutions and buffers

Use Saccomanno Fixation Solution and Oil Red O Solution. Harris Hematoxylin can be used as a counterstain. Prepare a 60% isopropanol solution with distilled water. All reagents are used at room temperature.

2

Wash the cells

Remove the cells from the incubator and carefully aspirate the medium. Gently wash the cells with 3 ml of Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/ Mg^{++} (Cat. No. C-40232). Repeat the wash step once more.

Note: Don´t disrupt the cell monolayer.

3

Fix the cells

Carefully aspirate the PBS and add 3 ml of Saccomanno Fixation Solution to the cell monolayer. Incubate at room temperature for at least 60 minutes.

4

Dilute the Oil Red O staining solution

During fixation, dilute 6 ml Oil Red O with 4 ml distilled water, then pass through a 0.22 µm syringe filter. Use within 30 minutes.

5

Wash the cells

Carefully aspirate the fixation solution and wash the cell monolayer with 3 ml of distilled water. Gently aspirate the water and repeat the wash step one more time. Afterwards, add 3 ml of 60% isopropanol to cover the cell monolayer. Incubate at room temperature for 3–5 minutes.

6

Add the staining solution

Carefully aspirate the 60% isopropanol and add 3 ml of diluted Oil Red O staining solution to cover the cell monolayer. Incubate at room temperature for 15–20 minutes.

7

Wash the cells

Carefully aspirate the staining solution and wash the cell monolayer several times with 3 ml of distilled water until the water is clear.

8

Counterstain cellular structures with Harris Hematoxylin staining

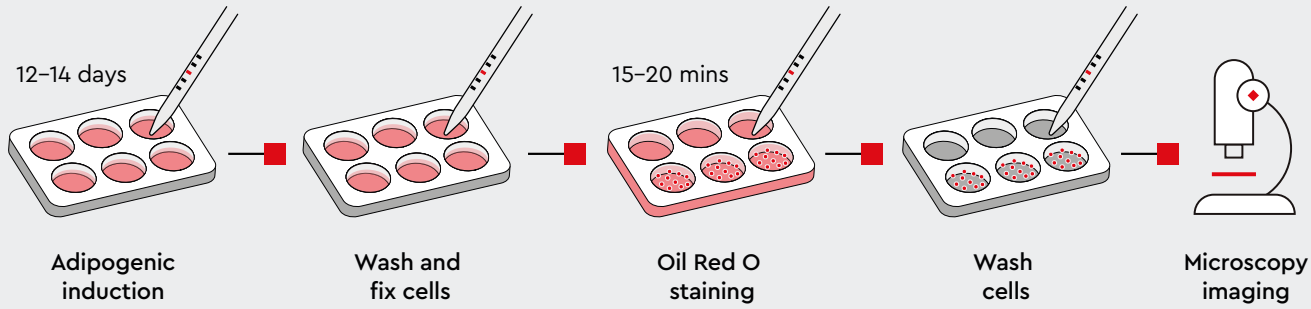
Filter the Harris Hematoxylin solution through cellulose paper, then add 3 ml of the filtered solution to each well. Incubate for 1 minute at room temperature. Wash the cells with 3 ml of distilled water until the water is clear.

9

Analyze the cells

Cover with PBS and analyze the stained samples under a microscope. Intracellular lipid vesicles in mature adipocytes will be stained bright orange to red (Fig. 3).

A



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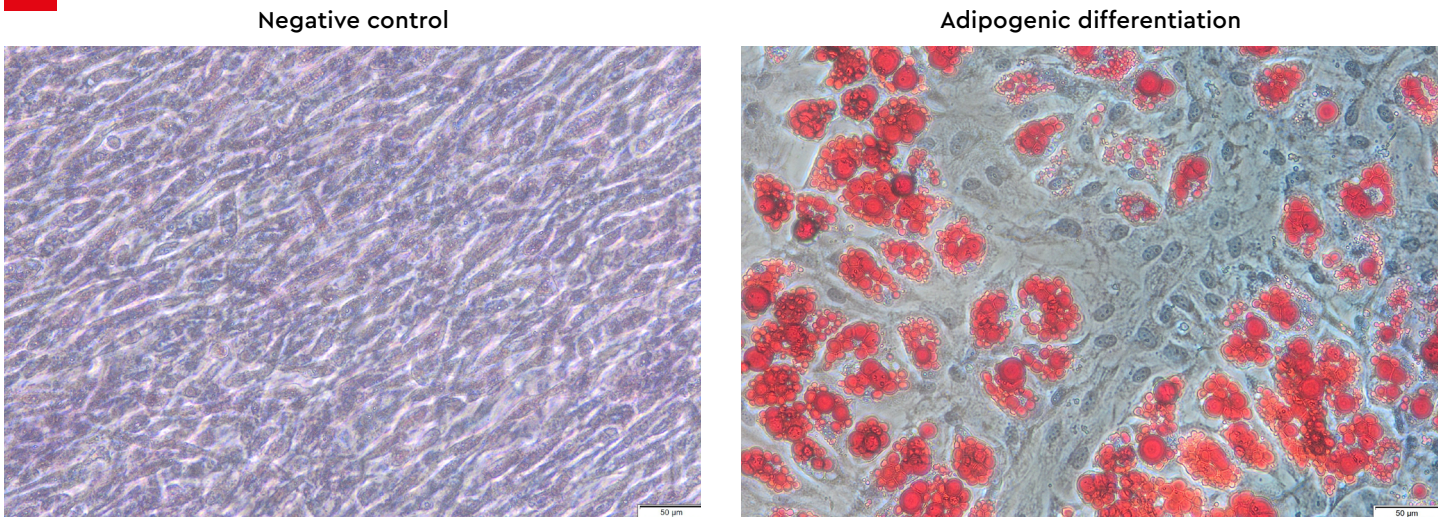

Fig. 3: Oil Red O staining of intracellular lipids in hMSC-BM-derived mature adipocytes. *A) Schematic overview of the adipogenic differentiation workflow, from MSC seeding through induction and staining. B) Representative microscopy images of hMSC-BM cultured for 12 days in Mesenchymal Stem Cell Growth Medium 2 (C-28009) as a negative control (left) or in Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (C-28016) for the differentiation sample (right). In contrast to the negative control, the mature adipocytes differentiated from MSCs exhibit intracellular lipid vesicles, as shown by bright red staining (scale bar, 50 µm). As a counterstain, Harris Hematoxylin was used to stain acidic structures, such as DNA and the endoplasmic reticulum, blue to purple.*

Key factors for Oil Red O staining	Description
Background	If the Oil Red O solution is not filtered properly or if it dries during staining, it can cause high background.
Differentiation	Suboptimal adipogenic differentiation negatively affects the staining quality and the number of lipid-positive cells.
Cell monolayer attachment	After two weeks in culture, cells may begin to detach. Handle gently during fixation and avoid forceful washing to preserve monolayer integrity.

Trademark references

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Products

Product	Size	Catalog number
Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)	500,000 cryopreserved cells	C-12974
	500,000 proliferating cells	C-12975
Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)	500,000 cryopreserved cells	C-12971
	500,000 proliferating cells	C-12972
Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)	500,000 cryopreserved cells	C-12977
	500,000 proliferating cells	C-12978
Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use)	500 ml	C-28009
Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use), phenol red-free	500 ml	C-28017
Mesenchymal Stem Cell Growth Medium XF (Ready-to-use)	500 ml	C-28019
Mesenchymal Stem Cell Growth Medium XF (Ready-to-use), phenol red-free	500 ml	C-28018
PromoExQ MSC Growth Medium 2, phenol red-free	500 ml	EQ-C-28017
PromoExQ MSC Growth Medium XF, phenol red-free	500 ml	EQ-C-28018
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 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, w/o Ca^{++}/ Mg^{++}	500 ml	C-40232

Additional products

Product	Catalog number
Falcon® Multiwell 6 Well	353046
Saccomanno Fixation Solution (Morphisto)	13881.00250
Oil Red O (Morphisto)	18575.00100
Harris Hematoxylin (Morphisto)	10222.00500

Contact us

Do you need support with experimental planning? Get in touch with our experts at scientific.support@promocell.com

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06/2026