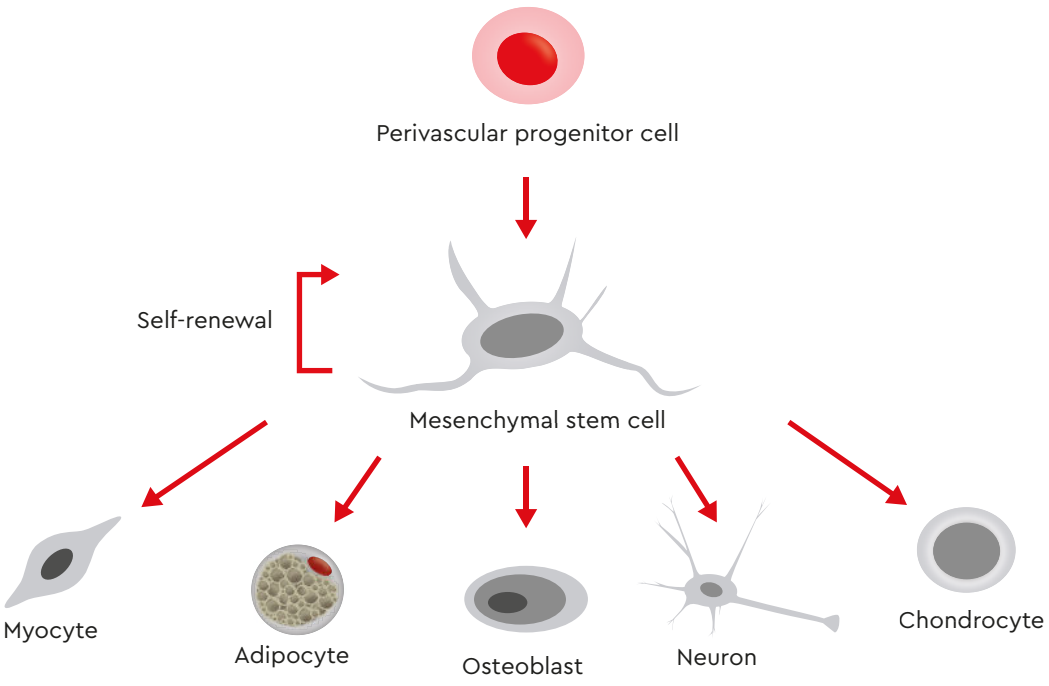
Chondrogenic differentiation and analysis of MSC

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 Sciety 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 $-\beta^+$
- Alkaline $Phosphatase^+$
- $CD31^-$
- CD34-

Mesenchymal stem cell

- $CD73^+$
- $CD90^+$
- $CD105^+$
- $CD14^-$ or $CD11b^-$
- $CD34^-$
- $CD45^-$
- $CD19^-$ or $CD79\alpha^-$
- HLA-DR-

Protocol for chondrogenic 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 Chondrogenic Differentiation Medium (C-28012)
- Dulbecco's Modified Eagle's Medium (DMEM, low- glucose) with 2 mM L-glutamine and 10% fetal calf serum
- Tissue culture treated 96-well U-bottom suspension culture plate

Use aseptic techniques and a laminar flow bench.

Chondrogenic differentiation of MSC is enabled by high density culture conditions, e.g. 3D spheroid cultures, in a chemical environment supportive of sustained differentiation. Our Mesenchymal Stem Cell Growth Medium 2 (C-28009) contains components likely to induce some degree of chondrogenic response when used on its own in combination with high density culture conditions. Therefore, the use of an alternative negative control medium in 3D spheroid cultures is recommended (see step 1).

1

Preparation of the negative control medium

The negative control medium is Dulbecco's Modified Eagle's Medium (DMEM, low- glucose) with 2 mM L-glutamine and 10% fetal calf serum.

2

Seed mesenchymal stem cells

Plate MSC at 2×10^5 cells per well in a 96-well U-bottom suspension culture plate using the negative control medium. Work in duplicate.

Note: The more cells you use, the larger the spheroids. Up to 3×10^5 cells per well can be plated.

3

MSC-spheroid formation

Spheroids will spontaneously form within 24–48 hours of incubation.

4

Induce MSC-spheroids

Induce one of the duplicate samples with MSC Chondrogenic Differentiation Medium (C-28012). Use the negative control medium for the remaining wells.

5

Differentiate induced MSC-spheroids

Incubate for 21 days. Change the medium every third day taking care not to aspirate the spheroids.

II. Detection of cartilage extracellular matrix

Materials

- Saccomanno Fixation Solution (Morphisto, Cat. No. 13881.00250)
- Ethanol (98–100%)
- Acetic acid (98–100%)
- Alcian Blue 8 GX
- 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.

Chondrogenic differentiation of MSC in 3D spheroid culture results in the formation of cartilage with its typical extracellular matrix. A key molecule within the cartilage matrix – besides collagen type II – is the proteoglycan, aggrecan. Aggrecan can be used as an indicator for cartilage formation and is stained dark blue using the copper-containing dye, Alcian Blue.

1

Prepare solutions and buffers

Use Saccomanno Fixation Solution (Morphisto, Cat. No. 13881.00250). Mix 60 ml ethanol (98–100%) with 40 ml acetic acid (98–100%). Dissolve 100 mg Alcian Blue 8 GX in this solution to prepare the Alcian Blue staining solution. This solution is stable for one year. To prepare the destaining solution mix 120 ml ethanol (98–100%) with 80 ml acetic acid (98–100%).

2

Wash the cartilage spheroids

Remove the cartilage spheroids from the incubator and carefully aspirate the medium. Carefully wash the spheroids twice with Dulbecco's Phosphate Buffered Saline (PBS) without Ca^{++}/ Mg^{++} (Cat. No. C-40232).

Note: Take care not to accidentially aspirate the spheroids.

3

Fixation of the cartilage spheroids

Carefully aspirate the PBS. Add enough Saccomanno Fixation Solution to cover the cartilage spheroids. Incubate at room temperature for 3 hours.

4

Wash the cartilage spheroids

Carefully aspirate the fixative and wash the spheroids twice with distilled water.

5

Stain the cells

Immediately before use, pass the required amount of Alcian Blue staining solution through a 0.22 µm syringe filter equipped with a PES-membrane. Carefully aspirate the distilled water and add enough filtered Alcian Blue staining solution to generously cover the cartilage spheroids, as some evaporation will occur. Incubate in the dark for 45 minutes at room temperature.

6

Wash the cells

Carefully aspirate the Alcian Blue staining solution and wash the cartilage spheroids with the destaining solution for 10 min. Repeat the wash step twice. Carefully aspirate the destaining solution and add PBS.

7

Analyze the cartilage spheroids

Cartilage will be stained an intense dark blue, whereas other tissue will, at most, stain light blue (see Fig. 1).

Note: Negative control spheroids from MSC cultured in MSC Growth Medium 2 may also stain light blue.

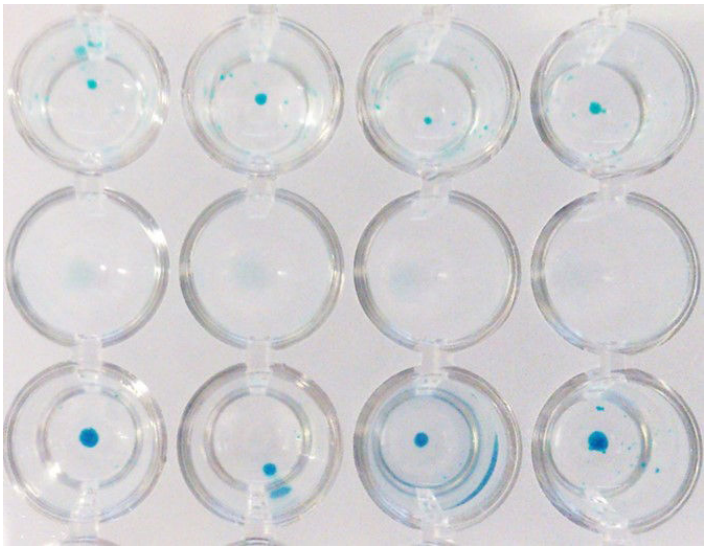


Fig. 1: MSC spheroids after in vitro differentiation into cartilage using our MSC Chondrogenic Differentiation Medium (stained for aggrecans using Alcian Blue). *Spheroids cultured in the negative control medium stain light blue (upper row). In contrast, the induced spheroids exhibit an intensely blue color indicative of cartilage extracellular matrix (lower row).*

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

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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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03/2023