How do Mesenchymal Stem Cells work?

Learn about MSC isolation, expansion and differentiation

What is a Mensenchymal Stem Cell (MSC)?

MSCs are fibroblastoid multipotent adult stem cells with a high capacity for self-renewal. These cells have been isolated from several human tissues, including bone marrow, adipose tissue, umbilical cord matrix, tendon, lung and periosteum [1, 2].

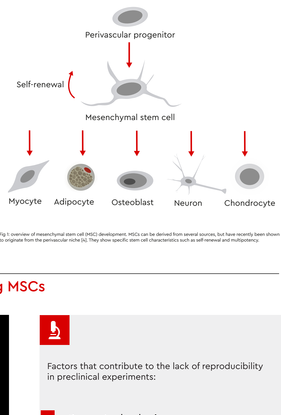

Fig 1: overview of mesenchymal stem cell (MSC) development. MSCs can be derived from several sources, but have recently been shown to originate from the perivascular niche [4]. They show specific stem cell characteristics such as self-renewal and multipotency.

Importance of characterizing MSCs

Scientists use different methods for cell isolation and expansion and take different approaches for characterizing their MSCs.

These inconsistencies make it difficult to determine whether the cells used in the experiments are similar enough for the results across different groups to be compared.

Factors that contribute to the lack of reproducibility in preclinical experiments:

1. Poor study design
2. Use of different biological reagents
3. Inconsistent reference materials

Criteria to identify MSCs

The International Society for Cellular Therapy (ISCT) has defined **three minimum criteria** for ensuring the integrity and unambiguous identification of human MSCs in order to provide a common set of comparable standard criteria for MSC research [3].

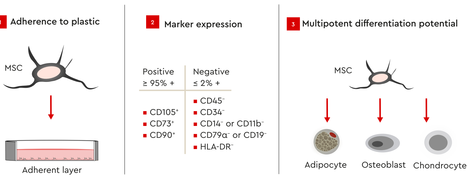

Fig. 2: summary of the ISCT criteria for identifying MSCs for research purposes. 1. MSCs must be plastic-adherent under standard culture conditions. 2. MSCs must express the surface antigens CD105, CD73 and CD90. A lack of expression of hematopoietic antigens (CD45, CD34, CD14/CD11b, CD79α/CD19, HLA-DR) is recommended, along with a minimum purity of ≥ 95% for CD105, CD73 and CD90 positive cells and ≤ 2% expression of hematopoietic antigens. 3. MSCs must be shown to be multipotent and be able to give rise to adipocytes, osteoblasts and chondrocytes under standard in vitro tissue culture-differentiating conditions.

List of cell surface antigens recommended by the ISCT for identifying MSCs:

Tab. 1

	Surface antigen	Alternative name	Cells expressing
Positive for	CD105	endoglin	Originally recognized by MAb SH2; endothelial cells, MSCs, hematopoietic cells
	CD73	ecto 5' nucleotidase	Originally recognized by MAb SH3 and SH4; B- and T-cell subsets, dendritic reticulum cells, epithelial and endothelial cells
	CD90	Thy-1	Hematopoietic cells, neuronal cells, fibroblasts, stromal cells, activated endothelial cells
Negative for	CD45	L-CA, PTPRC	Pan-leukocyte marker
	CD34	–	Primitive hematopoietic progenitors and endothelial cells
	CD14 / CD11b	– / integrin α-M, CR3A	Monocytes, macrophages, Langerhans cells and granulocytes / granulocytes, monocytes, natural killer cells, T- and B-cells and dendritic cells
	CD79α / CD19	MB1, IGA / –	B-cells / B-cells and follicular dendritic cells
	HLA-DR	–	Not expressed on MSCs, unless stimulated, e.g. by IFN-γ

Sources: ISCT (International Society for Cellular Therapy), Abcam Human CD Antigen Guide

Cell Identity

Characterization of MSC surface markers by flow cytometry, following isolation from bone marrow using **PromoCell's MSC Growth Medium**, showed a defined MSC population according to the recommended ISCT markers for determining MSC identity (positive markers in Fig. 3A and negative markers Fig. 3B.

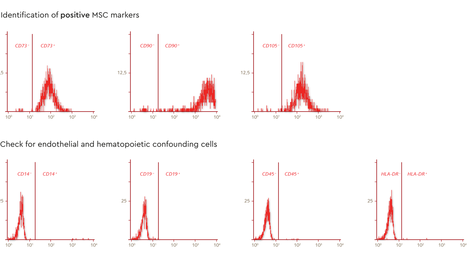

Fig. 3: Flow cytometry analysis of PromoCell human primary mesenchymal cells isolated from bone marrow. A. Histograms of CD73, CD90 and CD105 expression. MSCs show a defined population that is positive for the mentioned markers. B. Histograms of CD14, CD19, CD45 and HLA-DR exemplarily represent a defined population that is negative for the mentioned endothelial and hematopoietic markers. The results conform to the ISCT guidelines.

Self-renewal

Expansion of bone marrow-derived MSCs using PromoCell MSC Growth Medium DXF resulted in a stable growth performance over several passages (Fig. 4). The MSCs used in this investigation all meet the ISCT criteria (see Fig. 2), thus demonstrating the self-renewal capabilities of these defined cell populations.

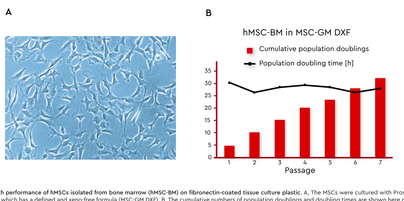

Fig. 4: Growth performance of hMSCs isolated from bone marrow (hMSC-BM) on fibronectin-coated tissue culture plastic. A. The MSCs were cultured with PromoCell Growth Medium DXF, which has a defined and xeno-free formula (MSC-GM DXF). B. The cumulative numbers of population doublings and doubling times are shown here over the course of 7 passages. A stable growth rate of less than 30 h/doubling can be observed even after prolonged in vitro culture for 32 population doublings over the course of 7 passages.

Multipotency

Differentiation of expanded bone marrow MSCs into adipocytes, chondrocytes, and osteoblasts, in accordance with ISCT criteria, was assayed in passage 3 using PromoCell MSC differentiation media (Fig. 5). All of the MSCs tested differentiated successfully into the three cell types, thus demonstrating their multipotency. Adipogenic differentiation exhibited the extensive intracellular lipid vacuole formation typical of mature adipocytes (Fig. 5, A). Chondrogenic differentiation of MSCs was determined by inducing cartilage spheroid formation (Fig. 5, B). Finally, differentiation of MSCs into mature osteoblasts was demonstrated by Alizarin Red S staining of extracellular calcium deposits in mineralized cells (Fig. 5, C).

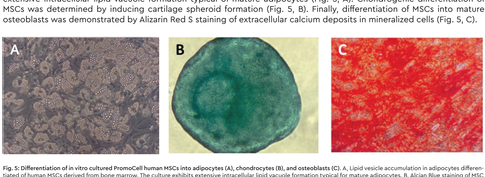

Fig. 5: Differentiation of in vitro cultured PromoCell human MSCs into adipocytes (A), chondrocytes (B), and osteoblasts (C). A. Lipid vesicle accumulation in adipocytes differentiated of human MSCs derived from bone marrow. The culture exhibits extensive intracellular lipid vacuole formation typical for mature adipocytes. B. Alcian Blue staining of MSC spheroids after in vitro differentiation into cartilage. Induced spheroids exhibit an intensely blue color indicative for cartilage extracellular matrix. C. Alizarin Red S staining of extracellular calcium deposits in mineralized hMSC-BM derived mature osteoblasts. The results are consistent with the 3rd criterion of the ISCT guidelines (see Fig. 2).

For more information, see the corresponding application note at www.promocell.com/downloads/application-notes.

References

[1] da Silva Meirelles L., Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. Stem Cells. 2008 26(9):2287–99.

[2 Ullah, I, Subbarao RB and Rho GJ. Human mesenchymal stem cells – current trends and future prospective. Bioscience Reports. 2015 35, e00191, BSR20150025

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[4] Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008 3(3):301–13.

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