Understanding
Mesenchymal Stem Cells (MSCs)

Stem Cell

Stem cells are primal cells found in all multicellular organisms and are defined by two characteristics:

- They are unspecialized cells that renew for a lifetime by cell division to maintain the stem cell pool
- They can differentiate into cells with special functions under particular physiologic or experimental conditions.

Mesenchymal Stem Cell (MSC)

Mesenchymal stem cells (MSC), which originate in the embryo from the mesodermal layer, can be found during all phases of the development of mammalians.

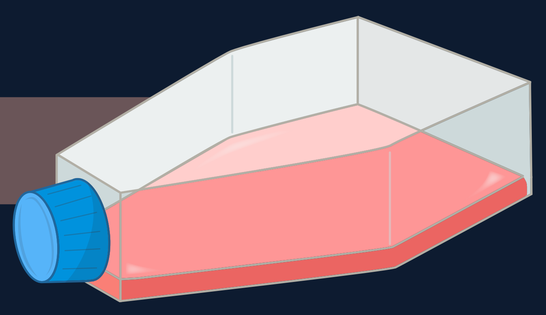
ISCT Guidelines to Define MSC

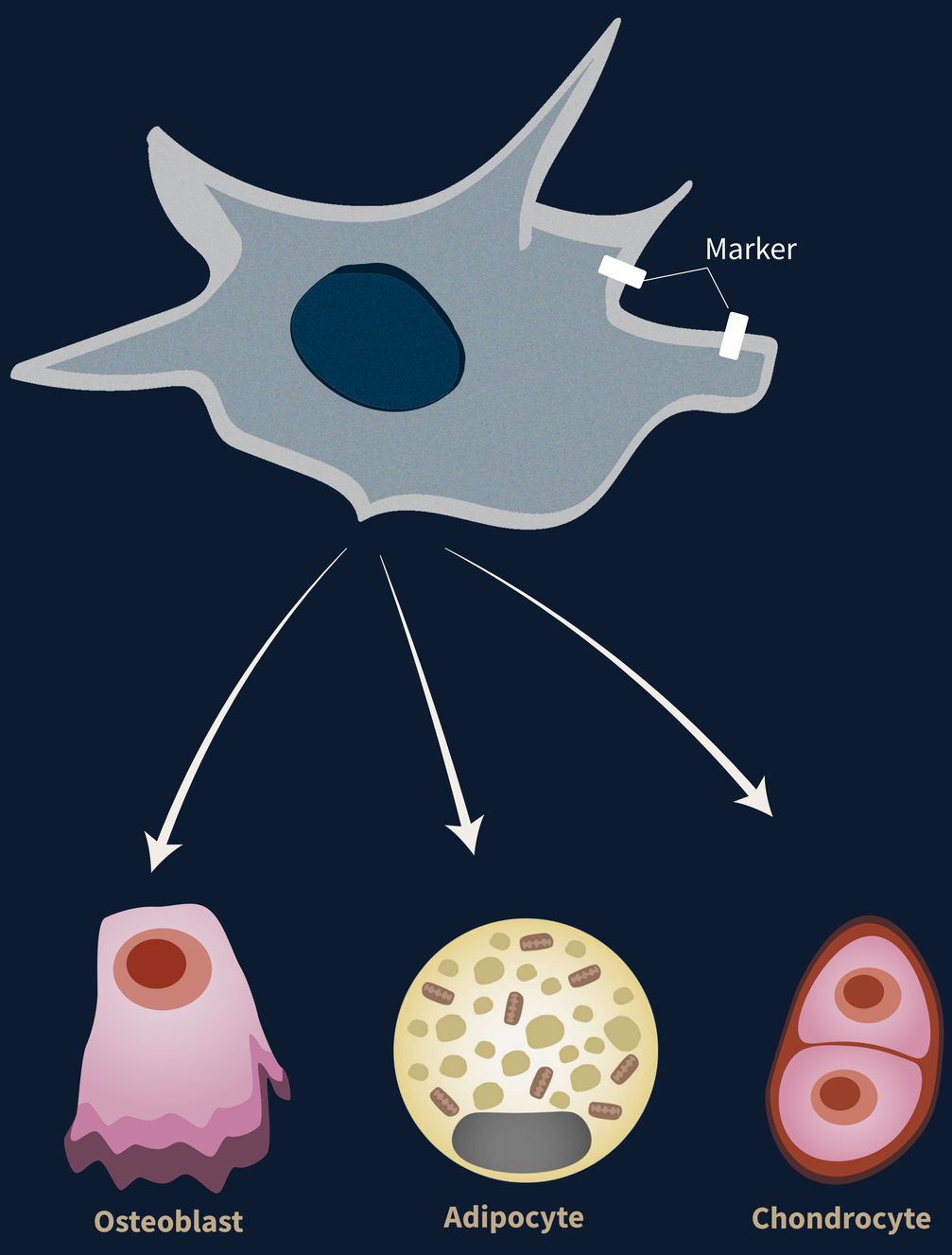
International Society ISCT Cell & Gene Therapy®

The International Society for Cellular Therapy (ISCT) has defined 3 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.

[1] Adherence to plastic

MSCs are adherence to plastic in standard culture conditions.





[2] Marker Expression

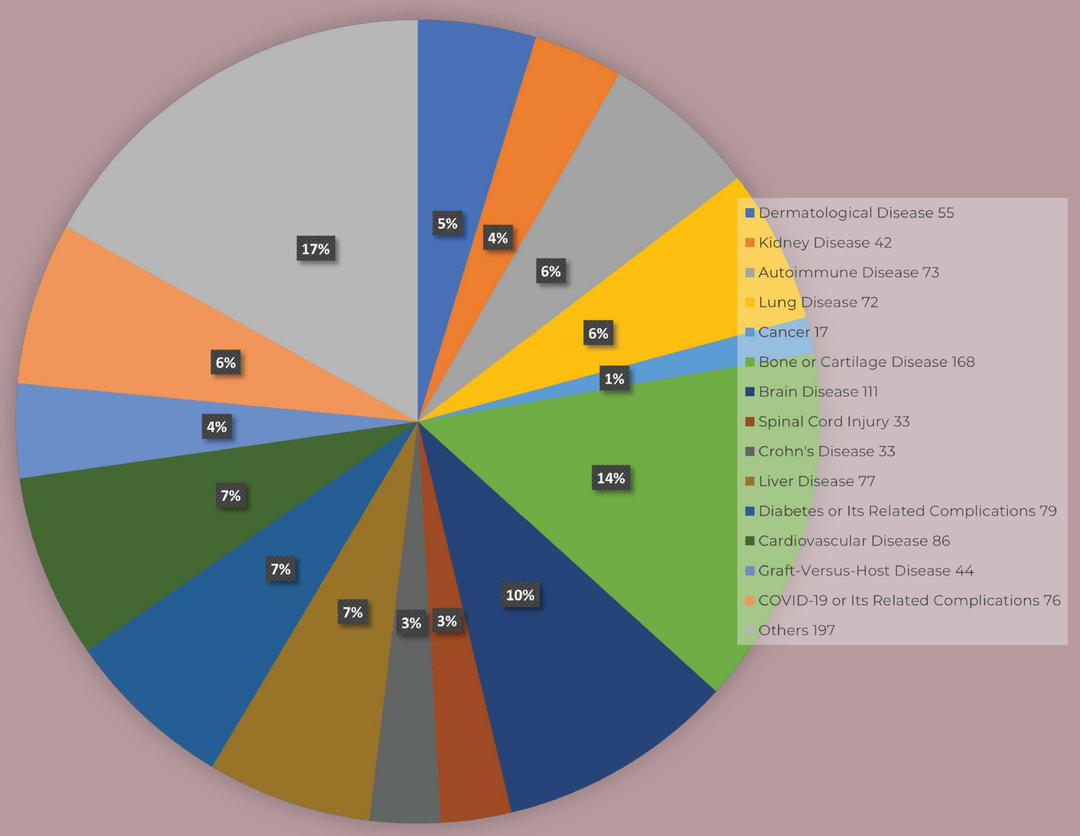
POSITIVE	CD105 CD73 CD90	**NEGATIVE**	CD45 CD34 CD14/CD11b CD79α/CD19 HLA DR

[3] Multipotent Differentiation Potential

MSCs are able to differentiate into cells of mesodermal lineages, including osteoblast, adipocyte and chondrocyte.

Clinical trials of MSCs are Classified by Disease Types

[Till 31st March 2022]
Data from: www. clinicaltrials.com



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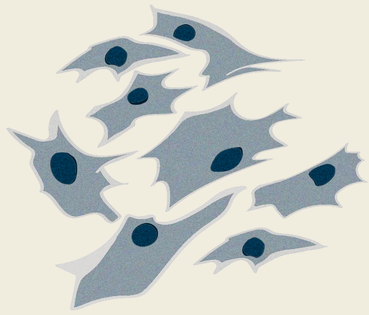
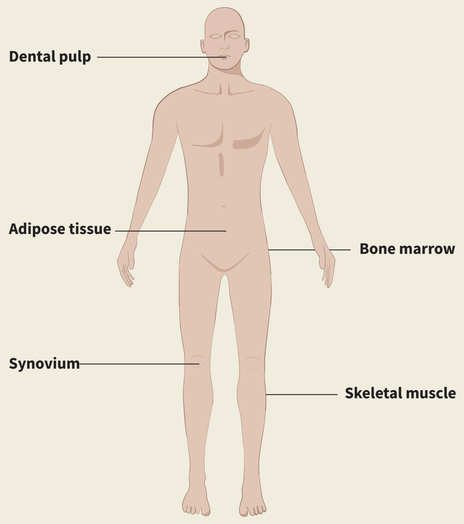
Biology of MSCs

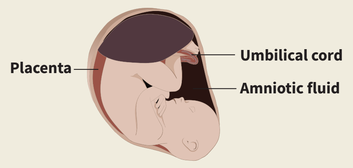


1 Cell Source

MSCs that isolated from **bone marrow (BM)** were the most common source used for the past clinical studies but the quantity of BM-MSCs in the human adult is very low (about 0.001-0.1%).

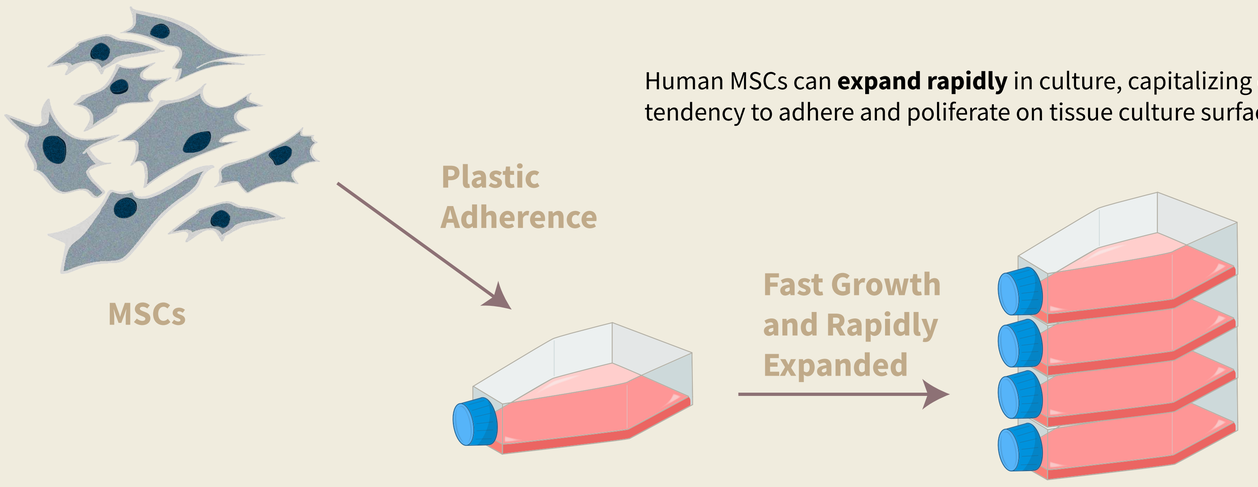
Subsequent studies have found that similar populations can be isolated from other adult and perinatal tissues. The cells derived from these diverse sources demonstrate very **similar characteristics** including cell marker expression, differentiation potential, and immunological properties.





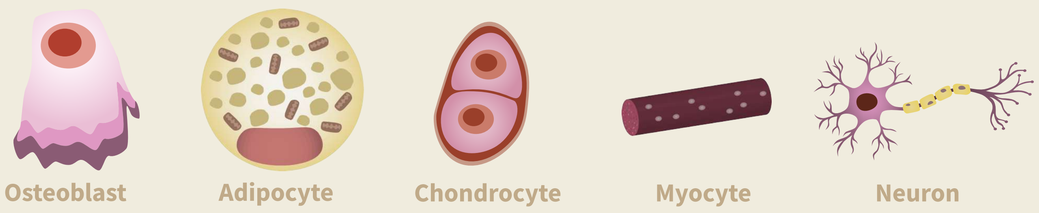
2 Rapid Culture Expansion

Human MSCs can **expand rapidly** in culture, capitalizing on their tendency to adhere and poliferate on tissue culture surfaces.



3 Multilineage Differentiation

MSCs are capable of **extensive self-renewal in an undifferentiated state**, but upon appropriate and specific stimuli are capable of **multipotent differentiation** towards various cell lineages (ectoderm, mesoderm, endoderm). Wnt-induced signals play an important role in regulating MSC differentiation.



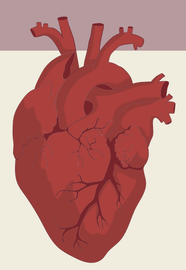
4 Paracrine Effects

MSCs actively **secrete cytokines and growth factors** that act either on themselves (autocrine function) or neighboring cells (paracrine function) to modulate the immune system, inflammatory responses, as well as apoptosis, fibrosis and angiogenesis.

- Immunomodulation
- Anti-Inflammation
- Anti-Apoptosis
- Anti-Fibrosis
- Angiogenesis

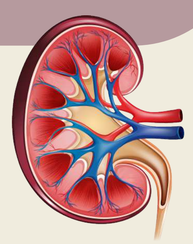
5 Clinical Applications

Chronic obstructive pulmonary disease (COPD)



Cardiovascular disease

Diabetes



Kidney disease

Autoimmune disease

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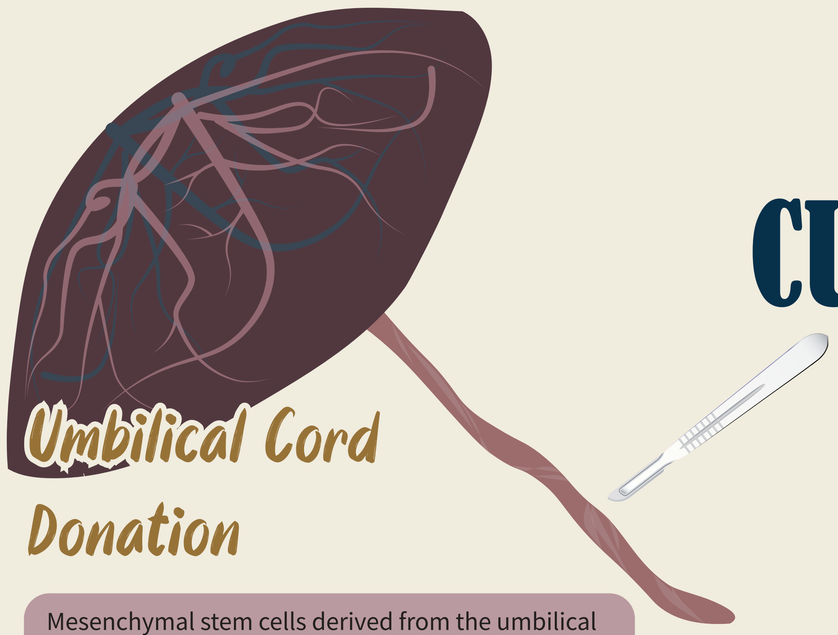
MSC CULTURING PROCESS



Umbilical Cord Donation

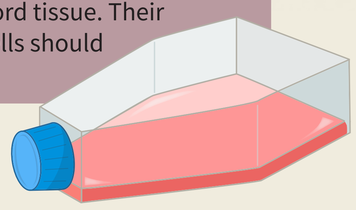
Mesenchymal stem cells derived from the umbilical cord vein (UC-MSCs) is an alternative sources of MSCs from bone marrow (BM-MSCs). Moreover, the procedure for UC-MSCs isolation is not invasive and since the cells are of fetal origin, their proliferative and differentiation potential could be better than that of MSCs from other sources.

Passaging of cells is a common procedure wherein cells from a given culture are divided, or “split”, into new cultures and fed with fresh media to facilitate further expansion.

MSC Isolation

Passage, P=0

MSCs are isolated from cord tisssue. Isolation methods, such as enzyme digestion and tissue explant, are available to isolate MSCs from cord tissue. Their efficiencies and cell growth of cells should be taken into consideration.



1. MSC Sources

For MSC products, the starting cellular material is arguably one of the most important determinants of product quality, efficacy, and safety. Therefore, specifications must be clearly defined for the MSC source. All allogeneic donors who meet selection criteria are subject to screening and testing measures aimed at preventing transmission of communicable diseases.

2. Anxiliary Materials

To isolate and expand MSCs, ancillary materials (AMs) that are not part of the final product formulation are used. The US Pharmacopeia, ICH and EU guidelines provide recommendations on the qualification, characterization, and testing of AMs to appropriate standards suitable for clinical use.

3. Quality Control

The MSC-manufacturing process, intermediate products and final product are controlled by testing for various safety (sterility and mycoplasma), identity, purity (endotoxin free), and potency attributes throughout the process and as part of lot release. Guidelines for safety and endotoxin testing are well-established and standardized between various jurisdictions

4. Excipients

Excipients are inactive ingredients added to the final product formulation to protect the active ingredient. Regulators require information on source, concentration, qualification, and characterization of all excipients, in a similar manner to AMs.

5. MSC Manufacturing Process

Cell manufacturing for clinical applications is unable to perform under a terminal sterilization step. This necessitates that the product be manufactured under strict aseptic conditions through the entire production process. Strict gowning practices, cleaning practices, and environmental monitoring (viable and nonviable) are critical for ensuring that the manufacturing environment is maintained in a controlled state during clinical production.

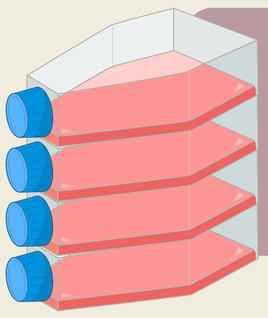
Multiple Aspects of MSC Culturing

GMP
Good Manufacturing Practices

MSC BIOPROCESSING

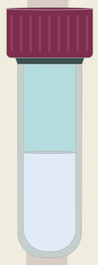
MSC Expansion

P=2



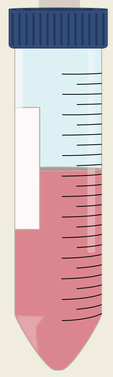
MSCs are plated (P=0) in cell culture flasks and incubated at 37 °C with 5% humidified CO_2 using the selected MSC culture media. 24 - 48 hours later, the non-adherent cells are removed and the adherent cells are expanded in culture with media changed every 3-4 days.

MCB Thaw



MSC Expansion

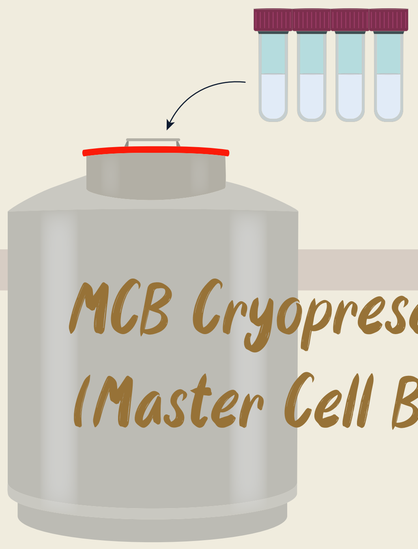
Cells from the seed bank (P = 2) are typically expanded through several additional passages to generate the final MSC product (P = 4–6) to be used in clinical trials.



P=4-6

Final MSCs

At this point of the manufacturing process, the MSCs may either be expanded directly to final product or expanded to an intermediate stage (P=2) where they are harvested and cryopreserved to create a seed bank for future production trials. The creation of MSC seed banks allows future production campaigns to be performed.



MCB Cryopreservation (Master Cell Bank)

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Mechanism of Action (MoA)
of Mesenchymal Stem Cells (MSCs)

MSC's regenerative properties and modulation of the immune system have driven their therapeutic application for a variety of conditions.

RECEPIENT CELL

PARADIGM SHIFT:
from Cell Replacement to Paracrine Provider

There are two major paradigms of tissue regeneration associated with MSC transplantation. First, the differentiation paradigms fueled prospects for cell replacement where damaged tissue could be readily renewed.

However, in the earliest studies stated that protection by MSCs from development of osteoarthritis, no implanted cells were detected in the cartilage but at the surface of regenerated menisci.

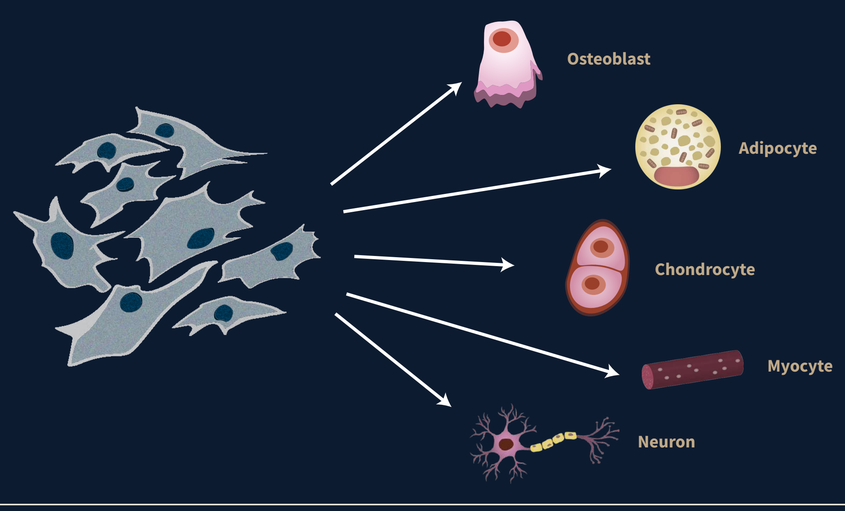
This paradigm was replaced by a second paradigm involving trophic mechanisms where the transplanted cells secrete immunomodulatory and repair factors that stimulate a host repair response. This paradigm was then confirmed by evident improvement in clinical trials.

EXOSOME

MESENCHYMAL STEM CELL

1 Differentiation

The first paradigm states that MSCs act as multipotent competent cells capable of migration, engraftment, and differentiation to the appropriate lineage, and that the newly formed tissue is populated by transplanted cells.

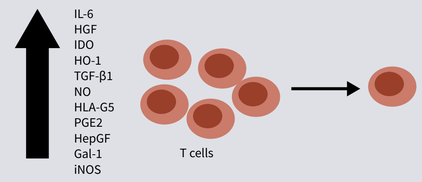


2 Paracrine Effects

Over the past decade the emphasis has shifted toward harnessing the MSCs' ability to produce factors and cytokines that stimulate innate tissue repair and modulate inflammation and immune responses. This paradigm was tested by many clinical trials for the paracrine activity of MSC.

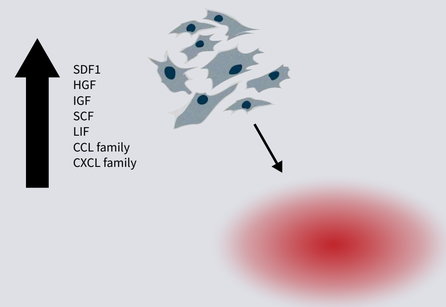
Immunomodulation

Immunomodulation is involving the change of body's immune system, caused by agents that activate or suppress its function. MSC produces at least 11 factors known to affect immune cells (dendritic cells, T cells, B cell, natural killer cells, macrophages).



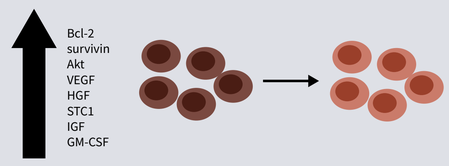
Cell Migration & Stimulation

MSCs showed faster mobilization and better retention at sites of injury following systemic or local intratissue infusion.



Anti-Apoptosis

To prevent programmed cell death, MSCs not only restore the blood flow but also synthesize and secrete proteins that are classic inhibitors of apoptosis, such as B-cell lymphoma 2 (Bcl-2), survivin, and Akt.

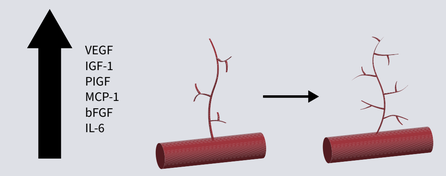


Anti-Fibrosis *(Anti-Scarring)*

Immunomodulation is involving the change of body's immune system, caused by agents that activate or suppress its function.

Angiogenesis

Angiogenesis is evidenced by formation of a new blood network from the preexisting capillaries by sprouting and proliferation. Angiogenesis is tightly regulated by a competitive balance of the angiopoietins and inhibitors, known as "angiogenic switch"



REFERENCES

[1] Peiman, H., & Viswanathan, S. (Eds.). (2016). Mesenchymal stromal cells: Translational pathways to clinical adoption. Academic Press.
[2] Turksen, K. (2013). Stem Cell Biology and Regenerative Medicine.
[3] Moll, G., Hoogduijn, M. J., & Ankrum, J. A. (2020). Safety, efficacy and mechanisms of action of mesenchymal stem cell therapies. Frontiers in Immunology, 11, 243.
[4] Liang, X., Ding, Y., Zhang, Y., Tse, H. F., & Lian, Q. (2014). Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. Cell transplantation, 23(9), 1045-1059.
[5] Pittenger, M. F., Discher, D. E., Péault, B. M., Phinney, D. G., Hare, J. M., & Caplan, A. I. (2019). Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regenerative medicine, 4(1), 1-15.
[6] Han, Y. F., Tao, R., Sun, T. J., Chai, J. K., Xu, G., & Liu, J. (2013). Optimization of human umbilical cord mesenchymal stem cell isolation and culture methods. Cytotechnology, 65(5), 819-827.
[7] Singer, N. G., & Caplan, A. I. (2011). Mesenchymal stem cells: mechanisms of inflammation. Annual Review of Pathology: Mechanisms of Disease, 6, 457-478.

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