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Mesenchymal Stem Cells: New Aspect in Cell-Based Regenerative Therapy

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ABSTRACT

MSCs are multipotent progenitors which reside in bone marrow. They support hematopoietic stem cells homing, self renewal and differentiation in bone marrow. They can also differentiate into osteoblasts, adipocytes, chondrocytes, myocyates and many other tissues. In vivo, when trauma happens, MSCs operate cell renewal and migrate to the damaged tissues to regenerate that injury. In vitro, MSCs are able to proliferate and differentiate to a variety of cell lineages. This makes them a very hopeful tool for cell-based regenerative therapy for large bone defects, maxillofacial skeletal reconstruction, cardiovascular and spinal cord injury and so many other defects. The most important characteristic that make MSCs an excellent tool for cell replacement is their ability to escape from immune rejection. For therapeutic purposes they usually isolated from human bone marrow or fat and they should proliferate in order to reach an adequate number for implantation. Conventionally DMEM medium supplemented with 10% FBS is used for their expansion, but currently autologous platelet rich products are replaced FBS. Platelet granules contain so many growth factors that can support MSCs proliferation.

Introduction

Two population of multipotent progenitors reside in bone marrow (BM): Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs).[1,2] In bone marrow MSCs are able to support hematopoiesis by releasing stromal-derived factor-1, Flt-3 ligand and stem cell factor.[3] This subset of cells can migrate to damaged tissues[4] and differentiate into at least three lineages: osteoblasts, adipocytes, and chondrocytes.[5-7] Trilineage differentiation is considered as minimal criteria for their multipotency.[8,9]

This class of multipotent progenitors first were recognized by Friedenstein et al in France in 1968.[10] Friedenstein and his colleges described an undifferentiated heterogeneous subset of cells which were spindle-shaped, plastic-adherent, non-phagocytic with fibroblast-like morphology.[11,12] Later in 1974, when they cultured a small amount of these cells in basal cell culture medium and saw their ability to generating clonal fibroblast-like colonies, they recognized that these cells had a high potential of proliferatition, therefore they named them fibroblast colony-forming cells (CFU-F),[11] afterward these cells entitled as mesenchymal stem cells.

In addition to bone marrow, MSCs can be identified in other tissues like fat, muscle, perichondrium, dental pulp and fetal tissues including BM, spleen, lung and liver;[13-15] as well as in other animals like mouse, rat, cat, dog and horse.[16] For experimental and therapeutic purposes, MSCs are usually isolated from human bone marrow and fat.[17,18] Although MSCs are characterized by their ability for differentiation into bone, fat, and cartilage,[5,6] they can also differentiate into other tissues including tendon, muscle, nerve, liver, kidney, pancreas and skin.[16,19]

Bone marrow-derived MSCs characteristically lack hematopoietic antigens including CD45, CD34, CD133, CD14, and MHC class II as well as endothelial antigens including CD80, CD34, CD31, vWF. On the other hand, they express stromal cell surface markers and adhesion molecules such as

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CD105, CD106, CD166, CD90, CD73, CD44, CD140b, CD9, CD10, CD13, and CD29.[1,20,21]

MSCs have a significant role in lymphopoiesis and myelopoiesis and show extensive immunomodulatory properties too. MSCs arrest B and T lymphocytes in G0/G1 phase of the cell cycle, thus inhibit their responses. MSCs can prevent monocytes from their function as an antigen-presenting cell (APC). Moreover, they improve regulatory T cell expansion.[22,23] In vitro, MSCs release IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15.[24] Self-renewal potential[25] and multipotency are MSCs's hallmarks.[12] Bone marrow-derived mesenchymal stem cells (BM-MSCs) are simply isolated from a small aspirate of bone marrow and are able to proliferate ex vivo and generate a variety of cell types.[26] In vitro, MSCs can proliferate and differentiate into a desired cell lineage using a specific medium[27] with a mixture of growth factors and other elements.[28] For instance, if MSCs cultured in the presence of dexamethasone, h-glycerophosphate and ascorbic acid, they will differentiate to osteoblasts.[26] Moreover, following implantation of cultured and expanded MSCs into the damaged tissues, they are able to differentiate into mature cells.[28] Additionally, MSCs which undergoes genetic modification in vitro can release particular growth factors and cytokines subsequent to tissue implantation.[29]

These last characteristics make MSCs a very promising candidate for clinical applications of autograft regenerating therapies in tissues damaged by trauma, aging or acute and chronic diseases.[28] Furthermore, MSCs are appropriate therapeutic tool to repair large bone defects[30,31] and maxillofacial skeletal reconstruction;[32] as well as cell replacement therapy in diabetes,[33] spinal cord injury,[34] cardiovascular,[35] neurological,[34] pulmonary[36] and immunological diseases.[23] Over and above, MSCs have a supportive function in co-transplantation with hematopoietic stem cells[17,18,37,38] by secreting stromal-derived factor-1[3] Flt-3 ligand and stem cell factor,[24] along with expressing extra-cellular matrix proteins including fibronectin, Laminin and vimentin[39] which have a crucial role in HSC homing in bone marrow niche.[38,40] One of the most important properties that make MSCs an excellent tool for cell-based therapeutic strategies is their ability to escape from immune rejection; therefore, HLA-matching is not that much important for their implantation and HLA-mismatched donors can be selected too.[22,23]

MSCs comprise a remarkably rare population of unfractionated bone marrow and most tissues like fat.[23] It is absolutely difficult to determine the exact number of MSCs in bone marrow, due to different techniques of bone marrow aspiration and MSC isolation. However, it is estimated that MSCs are about 0.001% of mononucleated cells in BM,[30] although the number of them decrease with age.[41] For clinical purposes we need a large-scale of MSCs, therefore amplification procedure must be done to generate plenty of cells.

Optimized condition for MSCs expansion consist of low glucose α-DMEM (Dulbecco's Modified Eagle Medium) as basal media, 10% fetal bovine serum (FBS) as protein supplement supporter and penicillin/streptomycin solution to prevent bacterial contaminations. As recommended by Meuleman et al, enriching media with some synthetic growth factors or anti-oxidants will decrease the instance between MSCs isolation and clinical application.[42] Nowadays, some guidelines suggest to avoid utilize animal derivatives like FBS, synthetic growth factors and allogeneic materials for therapeutic application since they raise the risks of pathogen transmission, infection and immune responses. Hence, autologous alternatives like platelet lysate(PL),[43,44] platelet rich plasma(PRP)[45-47] and platelet rich fibrin(PRF)[48-50] can be employed. These three products are rich in platelets[51] which contain a variety of growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), transforming growth factor (TGFb), platelet factor 4 (PF-4), and platelet-derived epidermal growth factor (PDEGF).[52] These growth factors enhance and accelerate MSCs proliferation in vitro and in vivo.[45,53]

Conclusion

MSCs are capable to expand in vitro and differentiate into a variety of cell lineages either in vitro or in vivo after implantation.[4-6] As a result they can be considered as a hopeful tool for cell-based replacement therapy.[26-33] For this purpose, first they should be expanded in vitro to reach an adequate number for clinical approaches. Conventionally we use FBS for their proliferation in cell culture medium.[39] Since FBS is a bovine derived additive and may cause infectious, prion transmission or may raise immune responses; it cannot be an appropriate supplement for cell culture medium when transplantation expanded MSCs is our final intention.[40] Because platelets have a variety of growth factors which may enhance MSCs proliferation, platelet rich products would be an excellent alternative for FBS replacement.[49] Nowadays using autologous platelet rich products including platelet lysate(PL),[43,44] platelet rich plasma(PRP)[45-47] and platelet rich fibrin(PRF)[48-50] for MSCs expansion become more general.[53] Employing autologous platelet rich products for MSCs expansion is a convenient, non-toxic, safe and cheap therapeutic method that promotes using MSCs for cell therapy.

Conflict of Interest

The authors report no conflicts of interest.

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