Mesenchymal Stem Cells for Tissue Engineering

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ABSTRACT

Mesenchymal stem cells (MSCs) have generated a promise as a potential source of cells for tissue engineering application due to their instrinsic ability to self-renew and differentiate into functional cell types. With their ability to differentiate into multiple cell phenotypes, mesenchymal stem cells offer the potential to regenerate entire tissue from a single cell source. Recent studies have demonstrated that MSCs were successfully used for bone repair, cartilage repair and soft tissue repair. This paper briefly outlines the biological characteristics of Mesenchymal stem cell and its application in tissue engineering field.

Keywords: mesenchymal stem cell, tissue engineering

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Tissue engineering is an interdisciplinary field that applies the principles of engineering and life science towards the development of functional substitutes that restore, maintain or improve tissue function[1]. The underlying principle involves the utilization of biocompatible scaffolds, cells and bioactive molecules to promote the differentiation and maturation of the cell type of interest. These components, when combined, form a tissue-engineered construct, which can function as the tissue replacement material[2]. One primary consideration in tissue engineered organ replacement is the choice of cells and the cell source. An ideal cell source for tissue engineering should have the capacity to proliferate and then differentiate in vitro, in a manner that can be reproducibly controlled.

Human body houses several types of progenitor cells that are capable of dividing many times, while also giving rise to daughter cells with more restricted development potentials. Eventually these cells differentiate and have specific phenotypic characteristics that contribute to their highly specialized function. Examples of such stem cells include embryonic stem cells, hematopoietic stem cells and mesenchymal stem cells [3]. Although the embryonic stem cells has pluripotent capability and can be propagated for more than two years with approximately 400 population doubling cycles while maintaining a normal karotype, it has legal and moral controversies concerning their use for clinical application [4].

Mesenchymal stem cells (MSCs) are present in a variety of tissues during human development, and in adults they are prevalent in bone marrow. They can be isolated and expanded with high efficiency, and induced to differentiate into multiple lineage under defined culture conditions. Along with their extensive capacity for self renewal, MSCs display a broad potential for generating diverse differentiated progenies [2]. With their ability to differentiate into multiple cell phenotypes, MSCs offer the potential to regenerate entire tissue from a single cell source and have recently gained attention for tissue engineering applications [5,6,7]. In various animal models, MSCs have been succesfully used for bone repair, cartilage repair and soft tissue repair [4,7].

Characteristics of mesenchymal stem cell

Mesenchymal stem cells (MSCs) are a heterogeneous population of stem/progenitor cells with multipotent capacity to differentiate into mesodermal and non-mesodermal cell lineages. MSCs reside primarily in the bone marrow, but also exist in other sites such as adipose tissue, periodontal ligament, deciduous teeth, hair follicles, scalp subcutaneous tissue, peripheral blood, liver, placenta, umbilical cord blood, and fetal tissues. It can be plated and enriched using standard cell culture techniques [5,7,8].

MSCs have a fibroblastic morphology in a monolayer culture and adhere to the tissue culture substrate (figure 1). They start proliferating and form fibroblastic-like cell clusters (fibroblast colony forming units, CFU-F), whose number depends on MSCs clonogenic potential of the cell source. After splited and expanded, the cells become more homogenous and may proliferate without differentiating up to 35-40 population doubling in culture. Immunodepletion methods involving CD34/CD45/CD11b may be used to generate purified MSCs preparation. Although not immortal, they have the ability to expand numerous times in culture while retaining their growth and multipotent potential [2,7].

The minimal requirement for a population of cells to qualify as MSCs, as suggested by *the International Society of the Isolated Cells in Culture*, is to meet the three criteria, including (i) the plastic adherence of the isolated cells in culture, (ii) the expression

of CD105, CD73, and CD90 in greater than 95% of the culture, and their lack of expression of markers including CD34, CD45, CD14 or CD11b, CD79a or CD19 and HLA-DR in greater than 95% of the culture, and (iii) the differentiation of the MSCs into bone, fat and cartilage [5].

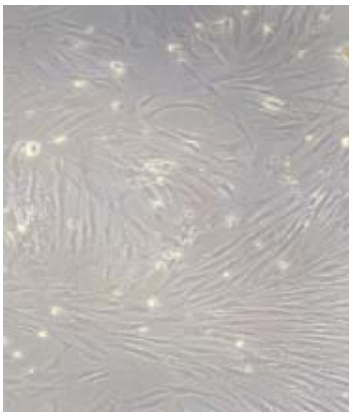

Figure 1. Human mesenchymal stem cells from bone marrow[2]

Differentiation of mesenchymal stem cells

MSCs multi-lineage differentiation potential has been extensively studied in vitro since their firs discovery in 1960s . Under defined inductive conditions, MSCs are able to acquire characteristics of cells derived from embryonic mesoderm, such as osteoblasts, chondrocytes, adipocytes, tendon cells, as well as cells possessing ectodermal and neuronal properties (figure 2). The lineage commited cells can fabricate a spectrum of specialized mesenchymal tissue including bone, cartilage, muscle, marrow stroma, tendon, ligament, fat and a variety of other connective tissues [3,9].

However, the molecular mechanism that gover MSCs differentiation are incompletely understood. Baksh et al, 2004, have proposed a model for the regulation of adult stem cell differentiation, wich incorporates two continuous yet distinct compartements (figure 3). In first compartement, MSCs undergo transcriptional modification, generating percusor cells without apparent changes in phenotype and self-renewal capacity. Similar to MSCs residing in adult bone marrow, the majority of MSCs cultured *in vitro* remain quiescent and growth arrested in G0/G1, until stimulated, for example, by the supplementation of growth factors. Upon stimulation, multipotent, uncommitted MSCs undergo asymmetric division, giving rise to two daughter cells, one being the exact replica of the mother cell and maintaining multilineage potential, and the other daughter cell becoming a precursor cell, with a more restricted developmental program. In this model, the precursor cell continues to divide symmetrically, generating more tripotent and bipotent precursor cells. These tripotent and bipotent precursor cells are morphologically similar to the multipotent MSCs, but differ in their gene transcription repertoire, and therefore, still reside in the stem cell compartment. The progression of MSCs to precursor cells is considered the first step in stem cell commitment. The transition or exit from the 'stem cell compartment' to the 'commitment compartment' occurs when precursor cells continue to divide symmetrically to generate unipotent progenitor cells, simultaneous with the acquisition of lineage specific properties, rendering them fully committed mature cells with distinguishable phenotypes. The commitment and differentiation of MSCs to specific mature cell types is a tightly and temporally controlled process, involving the activities of various transcription factors, growth factors, cytokines, and extracellular matrix molecules [9].

Application of MSCs in tissue engineering

MSCs present an progenitor cell source for applications of tissue engineering. It can be applied in the regeneration of bone, cartilage, soft tissue such as tendon, adipose and muscle. Combined with biocompatible scaffold and stimulated by specific growth factors, it construct biologic substitutes that will restore, maintain, and improve tissue functions following damage either by disease or traumatic processes.

Based on in vitro observation that MSCs can differentiate into osteocytes and chondrocytes, many attemps have been made to use expanded MSCs for in vivo tissue repair. For orthopedic applications, especially for bone formation, the use of natural or synthetic biomaterials have been used as carriers for MSCs delivery. As reviewed by Krampera et al[7], porous ceramic scaffolds loaded with in vitro expanded autologous bone-marrow derived MSCs were successfully implanted in 3 patients with large bone defect. An extended mandible discontinuity was successfully repaired through a heterotopic bone induction with biomaterials, patient's bone marrow and growth factors.

The use of synthetic polymers particularly PLA and PLGA, is also promising and applicable for the clinical reconstruction of joint cartilage defect. The induction of chondrogenic differentiation of bone marrow MSCs by tridimensional matrices and scaffolds has been studies in vivo in the presence of cytokines and recombinant human bone morphogenetic protein-2 (rhBMP-2). This approach that combines regenerating cells, bioactive matrices and osteoinductive growth factors seems to be mostly effective for the treatment of joint cartilage defect [7].

Induction of MSC differentiation into connective tissues other than bone and cartilage, such as tendons and ligaments, has been investigated for a potential clinical application.

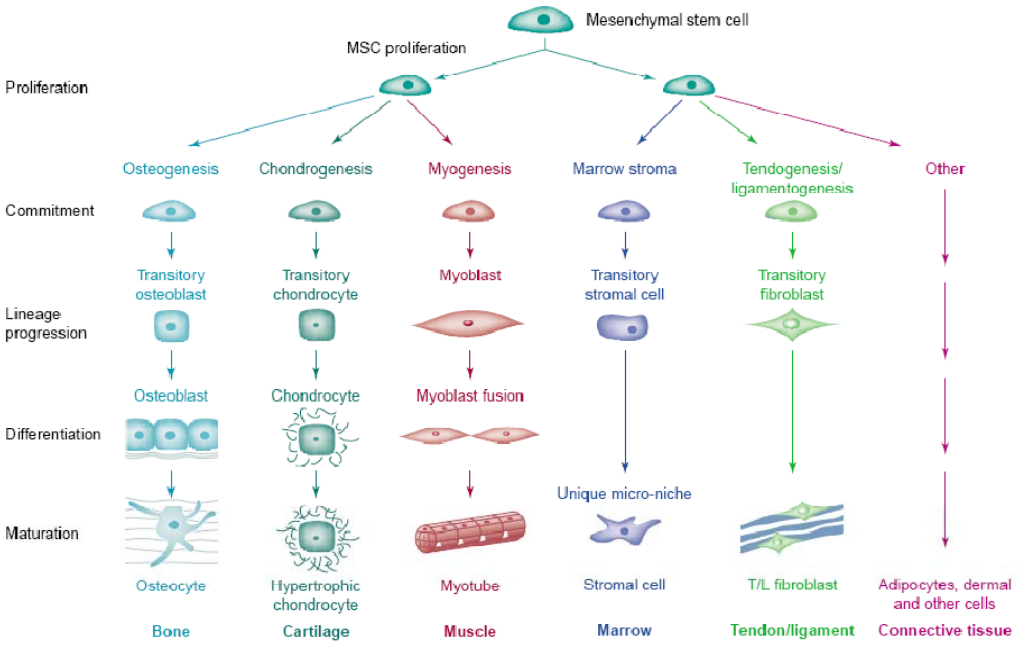

Figure 2. The stepwise cellular transitions from the putative MSCs to highly differentiated phenotypes are dipected schematically[3]

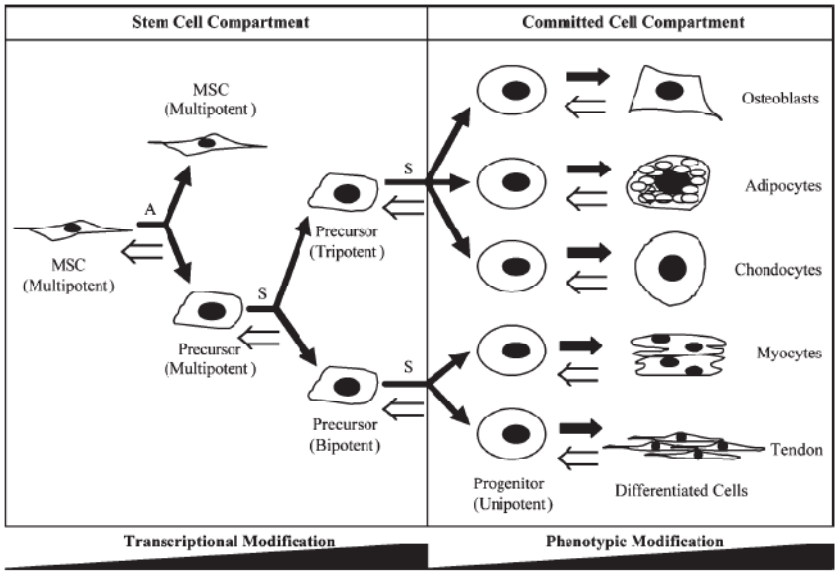

Figure 3. Schematic model depicting adult stem cell differentiation. Uncommitted MSCs undergo two stages, occurring in the stem cell compartment and the committed cell compartment, prior to acquiring specific phenotypes. In the stem cell compartment, multipotent MSCs give rise to a less potent cell population via asymmetric cell division (A), which then generate more precursor cells with less self-renewal capacity and more restricted differentiation potential via symmetric division (S). In the committed cell compartment, these tri-or bi-potent precursor cells continue to divide symmetrically and generate bi- or unipotent progenitor cells with pre-determined cell fate, which eventually give rise to fully differentiated cells. Recent studies also suggest that the fully committed cells are able to dedifferentiate into more potent cells, and acquire a different phenotype under inductive cues (open arrows) [9].

Induction of MSC differentiation into connective tissues other than bone and cartilage, such as tendons and ligaments, has been investigated for a potential clinical application. MSCs-collagen composites were implanted into long gap defects in the rabbit Achilles tendon: biochemical and histological analysis revealed an improvement of biomechanical properties, tissue architecture and functionality of the tendon after injury.At 12weeks post-surgery, the modulus and maximum stress for the repair tissue were 34% and 37%, respectively, of normal values. A further enhancement of tendon and ligament tissue regeneration derives from the use of exogenous growth/differentiation factors (GDF), for example, GDF-5, GDF-6 and GDF-7, which have been implicated in tendon formation [4,7].

CONCLUSION

Mesenchymal stem cells present an exciting progenitor cell source for applications of tissue engineering. Although much have been learnt about MSCs, a multidisiplinary approach still needed to discover more about the of using MSCs in tissue engineering.

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