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# Concise Review: Selecting the Source of Mesenchymal Stem Cells for Cartilage Regeneration Therapy

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## Abstract

Mesenchymal stem/stromal cells (MSCs) have been widely studied for regeneration therapy in various organs/diseases and are currently being developed for clinical practice. Despite the hope brought by MSC therapy, the characteristics of MSCs remain ambiguous, where cells have distinct features depending on their sources and species. With regard to cartilage therapy, MSCs from the bone marrow and synovium have been clinically examined based on their differentiation into chondrocytes in animal studies. However, recent studies have outlined other reparative mechanisms of MSCs, such as paracrine effects. Thus, the regeneration mechanisms are still elusive, and the key features of MSCs that determine their reparative activity have not been established. In this review, we summarize the current literature and discuss the importance of the assays to evaluate "human" MSCs considering the *in vivo* environment and reparative mechanisms.

**Keywords:** Mesenchymal stem cells • Bone marrow • Adipose tissue • Synovium • Cartilage • Regeneration therapy

## Introduction

The past few decades has witnessed a rapid growth in stem cell therapy using Mesenchymal Stem/Stromal Cells (MSCs). MSCs are found in the bone marrow; however, they can be isolated from several tissues, such as the adipose tissue, synovium, umbilical cord, and dental pulp, which are thought as medical waste. These abundant sources provide many patients with new therapeutic benefits for various diseases. Although many clinical trials have been conducted, little is known about the therapeutic mechanisms and optimal cell sources. Based on their differentiation potential, tissue engineering approaches were well studied especially in skeletal tissue regeneration. In this review, we introduce the general knowledge of MSCs and discuss skeptical facts between basic research and their clinical application for cartilage therapy.

## Literature Review

### Definition of MSCs

MSCs are multipotent cells with the capacity to self-renew, a feature that resembles fetal mesenchymal condensation [1]. In addition, they were shown to play an important role in maintaining homeostasis and tissue repair, such as bone remodeling and fracture healing, in postnatal life. However, the ability of endogenous MSCs to repair tissues through migration and differentiation is limited. The basic concept of MSC therapy is to supply enough MSCs to the injured site through *in vitro* purification and expansion.

The characteristics of MSCs were suggested in 2006 by Dominici, and include adherence to plastic, expression of specific surface antigens (positive for mesenchymal markers, such as CD105, CD90, and CD73, and negative for hematopoietic and endothelial markers, such as CD14, CD11b, CD19, CD79α, HLA-DR1, CD34, and CD45), and a potential to undergo multipotent differentiation *in vitro* into either osteogenic, chondrogenic, and adipogenic lineages [2]. However, given that MSCs and fibroblasts are phenotypically indistinguishable in their morphology, cell surface markers, differentiation potential, and immunomodulatory properties [3], the specified characteristics are not sufficient to interpret the uniqueness of MSCs. Moreover, the differential potential has been often assessed as discrete values, such as yes or no, whereas evaluating differential potential with continuous values, such as high or low, will yield further stratifying MSCs into various types based on the source, species, and culture method. Thus, a more precise definition of the characteristics of various MSC types is needed for future regeneration therapy.

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## Origins of MSCs

MSCs may be originated from different cell types which have distinct nature *in vivo*. In murine bone marrow, mesoderm-derived Nestin-negative MSCs and neural crest-derived Nestin-positive MSCs have been described [4]. Mesoderm-derived MSCs give rise to cells that contribute to the formation of chondrocytes and osteoblast precursors during the postnatal life. In contrast, neural crest-derived MSCs do not contribute to fetal endochondral development; however, they give rise to Schwann cell precursors, sympathetic nerve fibers, and postnatal MSCs. A distinct population of neural crest-derived MSCs in adults helps establish a niche for hematopoietic stem cells in the bone marrow by secreting the chemokine such as CXCL12. However, these functionally different stem cells showed comparable MSC features after being cultured *in vitro* [5].

The synovium, the connective tissue that lines the inner surface of the synovial joint capsules, is one of the sources of MSCs. Histologically; the human synovium is composed of several structures: the surface layer, stromal area, adipose tissue, and vasculature. Interestingly, all the components satisfied the criteria of MSCs after *in vitro* culture [6,7]. Although only some of them expressed MSC-specific surface antigens *in vivo*, there was no difference in the surface antigen expression *in vitro*. On the other hand, the potential for proliferation and chondrogenesis was superior in the perivascular region-derived MSCs, compared to that of others or bulk tissues [6].

Thus, various cell types can be transformed into MSC-like cells *in vitro*, with potentially distinct features and differentiation potential. Each donor site could include several sources of MSCs, which might result in irreproducible repair outcomes among patients. However, an adequate assay to select MSCs suitable for clinical practice remains missing.

## Mechanisms of MSC therapy

MSCs have been suggested as effective therapeutic agents for various diseases. It was initially hypothesized that transplanted/injected MSCs would migrate to injured or diseased sites, engraft, and differentiate into functional cells. However, an increasing number of studies have shown that MSCs repair damaged tissue by enhancing host cell viability and/or proliferation, reducing cell apoptosis, and modulating immune responses. Spees, et al. described alternative mechanisms of MSC-mediated tissue repair, including the paracrine effects of secreted growth factors, cytokines, and hormones; cell-cell interactions mediated by tunneling nanotubes; and release of extracellular vesicles [8]. These tissue repair capabilities and mechanisms led to the application of MSCs not only for skeletal injuries but also for various diseases, such as neurological diseases, cardiovascular diseases, graft-versus-host disease, hepatic cirrhosis, and lung diseases, including COVID-19 [9]. Some researchers have argued that mesenchymal "stem cells" is an inappropriate name considering their mechanisms. For instance, Caplan et al. stated that "medicinal signaling cells" represent the actual function of MSCs [10].

## MSCs for cartilage regeneration

Articular cartilage is formed at the end of epiphyses in the synovial joint cavity and is permanently responsible for the smooth movement of the synovial joint. Cartilage, as well as the central nerve and cardiac muscles are among the tissues known to have less reparative ability. As a tissue engineering approach for cartilage injuries associated with joint trauma, implantation of Autologous Cartilage or Chondrocyte-derived Tissue (ACI) to the injury site is conducted in current clinical practice. However, these methods exhibit some unresolved limitations, such as donor site morbidity and limited availability of chondrocytes. As an alternative cell source for chondrocytes, MSCs have been studied for their chondrogenic differentiation ability. Several studies have suggested that among MSCs, synovial MSCs (SMSCs) have a superior *in vitro* chondrogenic potential in humans and animals [11]. Based on the hypothesis on the relationship between the *in vitro* chondrogenic potential and *in vivo* cartilage regeneration capacity of SMSCs, clinical trials have been conducted to transplant SMSCs into the cartilage injury site [12,13]. However, repaired tissue contained fibrous tissue unlike articular cartilage; in addition, the superiority of SMSCs to ACI or other MSC types could not be proven. Furthermore, there are conflicting reports on the relationship between the chondrogenic potential of MSCs *in vitro* and *in vivo* among species. Koga et al. showed that Bone Marrow MSCs (BMSCs) and SMSCs had superior chondrogenic capacity *in vitro* in rabbits, which was also reproduced when they were locally transplanted into cartilage injury site [14,15]. Furthermore, the transplanted cells functioned as chondrocytes for over six months. Nakamura et al. found that SMSCs had the highest chondrogenic potential *in vitro* in porcine tissue; however, transplanted SMSCs did not engraft and disappeared within one month, resulting in cartilage repair to a lesser extent compared to the control non-treated animals [16]. Taken together, we suggest that the ability of MSCs to differentiate into chondrocytes *in vivo* is intrinsically different among MSC types and species, indicating that the *in vivo* environment/signaling should be considered when MSCs are locally transplanted without pre-induction.

## Chondrogenic conditioning of MSCs

The chondrogenic differentiation potential *in vitro* depends on differentiation-inducing growth factors and culture conditions. Transforming Growth Factor-β (TGFβ) and Bone Morphogenic Proteins (BMPs) are often used as chondrogenic differentiation factors. The combination of TGFβs and BMPs strongly induced chondrogenesis, compared with that when TGFβs or BMPs were used alone [17]. Moreover, other supplements, such as serum and glucocorticoids, could also affect chondrogenesis [17,18]. Dickhut et al. showed that when human BMSCs, SMSCs, and adipose-derived MSCs (AMSCs) were cultured with TGFβ3 without BMPs, only BMSCs showed full chondrogenesis, which was evident from the formation of tissue rich in hyaline cartilage components (type 2 collagen (COL2) and proteoglycan) *in vitro* [19] . Human SMSCs (SMSCs) and human AMSCs, in addition to TGFβ3, required BMP to give rise to a chondrogenic lineage. In addition, we showed that BMP2 alone or TGFβ3 + BMP2 induced full chondrogenesis in SMSCs; however, TGFβ3 alone induced incomplete chondrogenesis in SMSCs. The reactivity of these SMSCs was distinct among donors,where fibrocartilage-like tissue (COL2+/COL1+) was observed 1 of 3 donors and fibrous tissue formation (COL2-/COL1+) was observed for the other donors [18] . The chondrogenic potential of the SMSCs was shown to be superior when BMPs, not TGFβs alone, was used [11, 14-16].

Thus, conditions to increase the chondrogenic potential of MSCs are intrinsically different among MSCs, implying that the chondrogenic potential is probably misinterpreted depending on the assay conditions. Therefore, discovering *in vitro* signatures and/or assays that could reflect or predict the *in vivo* behavior of the transplanted MSCs is essential for the further development of MSC therapy.

## Response of MSCs to TGFβ is a key feature to predict the *in vivo* outcome after local transplantation.

Our recent study showed, for the first time, that the chondrogenic response of MSCs toTGFβ1 *in vitro* is a potential indicator for predicting the *in vivo* chondrogenic potential . [20] When AMSCs and SMSCs from mice or humans were transplanted into cartilage injury site in mice, different types of tissue repair were observed (Figure 1) . Transplantation of TGFβ1- reactive MSCs (mouse SMSCs and SMSCs from 1 of 3 human donors) resulted in chondrogenesis and cartilage-like tissue formation *in vivo*, whereas other MSC types resulted in fibrous tissue formation with engraftment at the transplantation site. However, mouse ASCs and SMSCs from all human donors showed chondrogenesis *in vitro* in response to BMP2 alone, which did not correlate with their *in vivo* chondrogenic potential. It has been previously reported that human BMSCs, which showed TGFβ1/3 -induced chondrogenesis, have the ability to repair osteochondral defects in rats by differentiating into hyaline cartilage-like tissue [18]. This finding suggests that our hypothesis might also apply to human BMSCs (Figure 1).

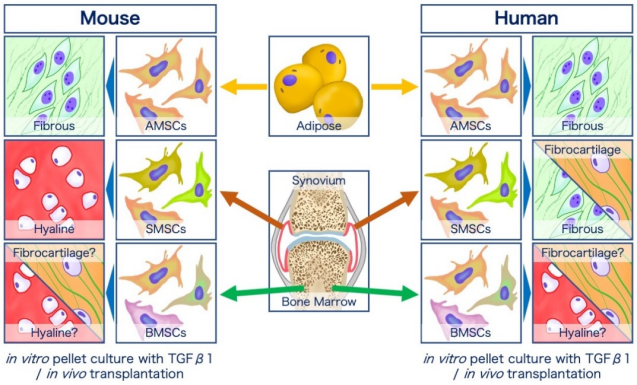


**Figure 1.** Outcomes of the transplantation of several mesenchymal stem/stromal cell (MSC) types into cartilage injury site. The chondrogenic potential *in vivo* correlates with their chondrogenic response to TGF β1 *in vitro*.

The role of TGFβ signaling is prominent in joint development and articular cartilage homeostasis [1]. In the synovial joint, chondrocytes and synoviocytes constantly replenish TGFβ, and high levels of TGFβ are present in the synovial fluid and cartilage extracellular matrix [21]. This might explain the importance of using TGFβ response to predict the chondrogenic potential *in vivo*. Although we initially thought that TGFβs present at the injury site might play an important role in the *in vivo* differentiation, the expression of TGFβ superfamily receptors and downstream Phospho-Smads in MSCs did not indicate the reactivity to TGFβ1. Therefore, we propose that the chondrogenic reactivity to TGFβ should be evaluated in culture assays, although more direct markers will be needed for future cell transplantation therapy.

In clinical practice, marrow-stimulation techniques can be used to treat cartilage defects. Bone marrow cells, including MSCs, can be introduced at the injury site, which subsequently promotes fibrocartilaginous repair. Therefore, it is expected that MSCs in the bone marrow differentiate into chondrocytes when stimulated with inducers, such as TGFβ, in the environment of human joints. With the exception of synovial chondromatosis, ectopic formation of cartilage tissue in the synovium or in adipose tissue has not been reported in general joint diseases (trauma, osteoarthritis, and rheumatoid arthritis). MSCs seem to maintain some of the characteristics of the original cells after *in vitro* expansion. Considering that most of their multipotency is not exerted *in vivo*, an *in vitro* differentiation assay for MSCs may be conducted with non-physiological stimulation.

## Conclusion

Because of the ambiguity of their definition, MSCs are sometimes pooled together, regardless of their origin. Certain paracrine signals might contribute to MSC differentiation, and some MSCs can differentiate into chondrocytes *in vivo* leading to tissue repair in response to the local environment. However, the chondrogenic ability of the MSCs varies depending on their characteristics, such as the tissue of origin, species, and donors. It is necessary to develop methods to identify and select the optimal MSC type depending on the disease.

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