

CURRENT CONCEPTS REVIEW

Mesenchymal or Maintenance Stem Cell & Understanding Their Role in Osteoarthritis of the Knee Joint: A Review Article

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Abstract

Mesenchymal Stem Cell (MSC) therapy in osteoarthritis has been hailed as a promising treatment for osteoarthritis due to their unlimited potential of healing and regeneration. Existing literature regarding their proper name, optimal sources, mechanisms of action, dosage, and route of administration, efficacy, and safety is debatable. This index review article has tried to connect these puzzling pieces of available information and brought clarity on some of these crucial issues. The author believes that Maintenance Stem Cells (MSC) may be a more suitable term than mesenchymal stem cell or medicinal signaling cells as their origin might not be limited to mesodermal tissue. Also, they have been shown capable of self-renewal, differentiation, and maintaining a cascade of healing & possibly regeneration at the implanted site. Only a small percentage of implanted MSC survive and rest undergo apoptosis after releasing growth factors, cytokines, and extracellular vesicles. These surviving MSC become active due to conformational changes induced by anti-environment stimuli and undergo limited self-renewal, proliferation, and differentiation, but only a few of them might incorporate into the host tissues. These cells generate & maintain a momentum of series of regenerative activities to improve the function of joint, stabilize or possibly enhance the cartilage quality. More randomized studies with long term follow-up are required to bring clarity on their ideal source, expansion, culture technique, optimum dosage, and route of administration and long-term safety issues.

Level of evidence: V

Keywords: Knee, Maintenance stem cell, Mesenchymal, Osteoarthritis

Nomenclature: Mesenchymal Stem Cell or Maintenance Stem Cell?

Caplan gave the current popular mesenchymal stem cell (MSC) term because of their mesenchymal origin and in vitro multipotency and clonability (1). Several clinical and research papers on these cells have been published over the last few decades. Many of these studies supported the In- vitro, In- vivo multipotency and clonability of mesenchymal stem cells retrieved from variable sources including bone marrow, adipose tissue, dental pulp, dermis and myocardium (2). These variable

sources, different retrieval & culture methods created a need to have universally accepted recommendations for the exact characterization of mesenchymal stem cells; also, questions were raised about their stemness at various platforms. International society for cellular therapy tried to sort out the issue of characterization and stemness of mesenchymal stem cells by giving their guidelines. They proposed that mesenchymal stem cells are not stem cells but are stromal cells, and they should be called a mesenchymal stromal cell (3, 4). According to

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their guideline, these cells must show plastic adherence in standard culture medium using tissue culture flask. Secondly, more than 95% of these must be positive for CD105, CD73, CD 90 and negative (< 2% positivity) for CD45, CD34, CD14 or CD11b, CD79a or CD 19 and HLA class II. Finally, they must be able to differentiate to osteoblast, adipocytes, and chondrocytes under standard In-vitro differentiating conditions (3, 4).

In 2017, Caplan revisited his initial description of mesenchymal stem cells and accepted the guideline that it is not stem cell; stem cell should show serial transplantation and double by cell renewal (5). However, he did not agree with the concept that MSC is derived from the connective tissue layer of different tissue (5). Because MSC cells are believed to act by secreting growth factors & cytokines which promote healing at the site of injury, inflammation, or diseased tissue, he changed the name of Mesenchymal Stem Cell to Medicinal Signalling Cells (5). Despite his recommendation, current literature and many ongoing clinical trials still use term Mesenchymal Stem Cell and believe in its stemness, but how it works as a stem cell is yet to be determined.

The author believes that Maintenance Stem Cells (MSC) may be a more suitable term than mesenchymal stem cell or medicinal signaling cells, as they might not be limited to tissues of mesodermal origin and once implanted, they maintain a cascade of healing & possibly regeneration. Only a small percentage of implanted MSC survive and rest undergo apoptosis after releasing growth factors, cytokines, and extracellular vesicles. These surviving MSC become active due to conformational changes induced by anti-environment stimuli and undergo limited self-renewal and proliferation but might not differentiate & incorporate into the host tissue or chondrocytes. These cells generate & maintain a momentum of a series of regenerative activities to improve the function of the joint and stabilize or possibly enhance the cartilage quality.

Sources of Mesenchymal Stem Cells

These cells were initially retrieved from bone marrow, adipose tissue, and umbilical cord. Now they are being said to be present in a variety of tissue, and organ-like synovium, placenta, amnion, umbilical cord, dental pulp, blood vessels, peripheral blood, and with more research, and this list is further going the increase in future.

Bone Marrow

It is one of the oldest and most prevalent methods to harvest stem cells for Orthopaedic and non- Orthopaedic indications. It has smooth and simple access, although cells have more osteogenic and chondrogenic differentiation potential than adipose tissue-derived stem cells, the percentage of stem cells may be less (1 in 25000 to 1 in 100,000) than the adipose tissue (6-9). The bone marrow-derived stem cell shows better viability & quality in the colony-forming unit than the adipose origin of stem cells. It may be done under local anesthesia but requires sedation in patients having anxiety or low pain threshold and has a mild risk of local infection.

Subcutaneous Adipose Tissue

It also has easy access from the abdomen and buttock; again, the number of MSC cells (10) constitutes 2 % of the total cells in the lipoaspirate; and has 500 times more pluripotent cells than in the equal amount of bone marrow. However, quality, viability, and osteogenic (same or superior one over the other) potential amongst the bone marrow and adipose-derived stem cell-derived is debatable (11, 12). The differences could be because of the age difference (also conflicting), different cell donors, harvesting, and culture methods (13-16).

Infrapatellar/Suprapatellar Fat Pad

Access to these sites is more invasive and is available during arthroscopic or open knee surgeries. While cells from the infrapatellar fat pad are more chondrogenic; cells from suprapatellar fat pad have shown to be more osteogenic & chondrogenic than bone marrow or subcutaneous adipose-derived stem cells in In-vivo & In -vitro studies (17-20).

Synovium

They are believed to have low osteogenic, better chondrogenic and high proliferation activity than cells retrieved from bone marrow or adipose tissue; Also, in comparison to bone marrow or adipose-derived MSC, they are said to retain their multi-lineage differentiation potential even with advancing age and during in-vitro expansion over four or more subcultures (21, 22).

Umbilical Cord

Some studies have shown that mesenchymal stem cell retrieved from umbilical cord might be a better alternative as they provide a higher number of cells from allogenic sources; avoid autologous harvesting related complications and has better clonogenic, proliferation, migration & chondrogenic potential than mesenchymal stem cell retrieved from bone marrow (23, 24). However, they are allogenic & which part of the umbilical cord provides the best source of stem cells is still debatable, more studies are required to document their real potential and problems.

Dental Pulp

It is another good source of mesenchymal stem cell producing mineralized tissue, extracellular matrix, dentin, dental pulp, and periodontal ligament in xenograft model studies (25, 26).

How Mesenchymal Stem Cells (MSC) works?

Mesenchymal stem cells are believed to work by paracrine & juxtacrine signaling and to exhibit anti-inflammatory, anti-fibrotic (bFGF, AMD, HGF), angiogenic, mitogenic, anti-apoptotic and immunoregulatory properties (PGE2, TSG-6, ECVs) (27-33). However, there is still uncertainty about their stemness, and if they are a stem cell, how they work when injected or applied in healthy or pathological sites.

Local injection or implantation of MSC into the target joint or tissues have been shown to have a low potential for overall homing; short stay at the implanted site (up

to 10 days) and low engraftment or incorporation (34-38). Even after intravenous injection of MSC, only a small number of implanted MSC cells have been found to home in healthy tissues, bone marrow, veins in the lung, and other target tissues (37).

The Author based on a review of the current existing literature proposes that Maintenance Stem Cell (MSC) may be a more suitable term than mesenchymal stem cell or medicinal signaling cells as their origin might not be limited to mesodermal tissue, they are capable of self-renewal and differentiation into other cell lineages like osteoblast, adipocyte & chondrocytes. They change anti-environment (pathological) into pro-environment (healing) and maintain this momentum of healing and regeneration by releasing pro-environment factors.

Proposed mechanism of Mesenchymal/Maintenance Stem Cell (MSC) in osteoarthritis

The proposed mechanism has attempted to connect all the available information with the author's views for decoding the functioning of MSC. In osteoarthritis, innate immunity (macrophages, mast cells, natural killer cells) plays a significant role in starting the pathogenesis of osteoarthritis while acquired immunity (B & T cells) establishes it as a chronic debilitating joint & synovial pathology (33). The anti-environment (catabolic) created by an increased level of IL-1, TNF- α & matrix metalloproteinase-13 lead to an increased level of IL-8, IL-6, leukotriene inhibiting factor, proteases and prostaglandin E2 (PGE2) which causes cartilage matrix degradation and osteoarthritis of the knee (33, 39-41).

When implanted in an anti-environment (catabolic), Mesenchymal Stem Cell starts the following series of three responses (Figure 1-3) to promote healing, regeneration, and repair.

First Induction Response (FIR)

Anti-environment of osteoarthritis, injured or degenerated joints, ligaments, tendon, and other tissues are pro-inflammatory, anti-angiogenic, apoptotic, and promote fibrosis. In this Anti-environment, Local Resident Stem Cells (LRSC) are active, but they are weak and do not have enough supply of energy and local support of growth factors and cytokines. When MSC are implanted or injected in this environment, they can attach the local collagen, laminin, fibronectin, and other extracellular matrix proteins (42). However, only a small number of MSC survive, and the rest of them undergo apoptosis after releasing growth factors, cytokines, and extracellular vesicles. Remaining surviving MSC becomes active (34) due to conformational changes in their surface receptors due to anti-environment stimuli (hypoxia, injury, TNF α). This phenomenon gets support from studies which have shown that freshly collected peripheral blood stem cell shows more MSC marker in hypoxic condition and after subcutaneous administration of human granulocyte colony-stimulating factor (43, 44).

These cells first facilitate to create a pro-growth environment by secretion (paracrine & juxtacrine) of growth factors, cytokines & Extracellular Vesicles (EVs), which starts inhibiting the pro-inflammatory

cytokines, anti-angiogenic factors, apoptotic and fibrotic factors. MSC produces TNF α -stimulated gene/protein 6 (TSG-6), prostaglandin E2 (PGE2) & indoleamine 2,3-dioxygenase (IDO); these promote the conversion of M1 macrophage (pro-inflammatory) to M2 macrophage (immunosuppressive) and reduce proliferation & cytotoxicity of Natural killer cells (33). TSG-6 (45, 46) also works through CD44 on macrophages and reduces the generation of inflammatory mediators (nitric oxide, TNF- α , and IL-1 35-38). MSC also prevent mast cell degranulation and production of TNF- α . MSC also creates an immunosuppressive environment by blocking IL-10 and TGF- β secretion (47, 48), which inhibits macrophages from the release of IL-1 β and TNF- α facilitated by lipopolysaccharide (49). The immunosuppressive effect of MSC also involves inhibition of PGE2, leading to the prevention of INF- γ stimulated T cell proliferation and increased expression of IL-6 and IL-10 to prevent macrophage differentiation towards dendritic cells (50). Furthermore, MSC also extends their immunosuppressive effects by preventing the division of B cells during G0 and G1 phases (51, 52). Pro-environment (anti-inflammatory/immunosuppressive) created by MSC may lead to the empowerment of existing weak but metabolically active & responsive local chondrocyte (53).

These surviving implanted MSC also undergo limited self-renewal, proliferation, and differentiation. Although all of these new MSC or differentiated cells might not incorporate into the host chondrocytes or chondroblasts, they can generate & maintain a regenerative environment to improve or maintain the cartilage quality (54, 55). Besides the above-mentioned anti-inflammatory & immunosuppressive effects of MSC, another critical function of MSC is to release the exosomes, which provide positive signals, energy, and support to the local weak resident stem cells (LRSC) (56, 57). As a result of this MSC support, LRSC becomes more active and follows the implanted stem cell to release more pro-environment growth factors, cytokines, extracellular vesicles, and also undergo limited self-renewal, proliferation, differentiation, and incorporate into local host tissues, chondrocyte/chondroblast gradually [Figure 1].

Sustained Induction Response (SIR)

SIR follows the First Induction Response; during this phase, pro-environment is further augmented by sustained release of more pro-growth factors from implanted MSC and LRSC, ongoing induction of the LRSC, and recruitment of more cells from limited self-renewed of LRSC and implanted MSC [Figure 2].

Limited Induction Response (LIR)

Soon after implantation, the majority of these MSC undergo apoptosis after releasing pro-growth factors and stimulation of LRSC; they are also joined by apoptosis of the remaining replicated, proliferated and differentiated MSC. This may be the reason that most of the implanted MSC disappear in a short time, and studies using tagged MSC implantation are not able to detect them in target host tissue (54). Apoptosis of these MSC lead to reduced

Healthy Tissue: Pro-Environment > Anti-Environment**Pro-Environment:**

- Anti-apoptotic
- Anti-inflammatory
- Anti-fibrotic
- Angiogenic

Local Resident Stem Cells:

Active and fully functional.

Anti-Environment:

- Apoptotic
- Proinflammatory
- Fibrotic
- Anti-angiogenic

Local Resident Stem Cells: Active but weak & exhausted.

Unhealthy Tissue (Age, trauma, disease, Congenital): Anti-Environment > Pro-Environment**Local Resident Stem cells:** Are weak

- Less energy reserves
- Less secretion of pro-growth factors/cytokine
- Reduced capacity of self-renewal/proliferation/differentiation
- Apoptotic

First Induction Response (FIR): Upon implantation only a small percentage of MSC survive and rest undergo apoptosis after releasing growth factors, cytokines, extracellular vesicles. Remaining MSC gets activated by anti-environmental stimuli and starts First Induction Response.

- Facilitate Pro-growth environment (F):** Secretion of growth factors, cytokines, chemokines. Inhibition of inflammatory cytokines, antigrowth, apoptotic factors (Paracrine/Juxtacrine).
- Inducing Local Resident Stem Cell (LRSC):** Extracellular Vesicles supply signals, energy and local support to LRSC & induce LRSC to follow MSC function.
- Recruitment of new MSC & LRSC:** Although remaining MSC undergo limited self-renewal, proliferation, differentiation and might not incorporate into the host tissue, chondrocyte/chondroblast; however, they induce LRSC to undergo limited self-renewal, proliferation and differentiation to maintain pro-environment.

Figure 1. Proposed Mechanism of Mesenchymal/Maintenance Stem Cell (MSC):

First Induction Response (FIR): Stemness, Paracrine, Juxtacrine.

Environment: Pro-Environment > Anti-Environment

Mediators: Mesenchymal Stem Cells + Local Resident Stem Cells + Growth Factors/Cytokines

MSC:

S: Sustained release of growth factors, cytokines, exosomes

I: Induction of LRSC

R: Recruitment of new MSC & LRSC Cell

Local Resident Stem Cell (LRSC) are active:

Increased numbers (Self renewal)

Increased Energy reserve: Exosomes from MSC

Increased secretion of pro-growth factors/cytokines

Less apoptotic potentials

Pro-environment:

Anti-apoptotic

Anti-inflammatory

Anti-fibrotic

Angiogenic

Figure 2. Proposed Mechanism of Mesenchymal/ Maintenance Stem Cell (MSC): Sustained Induction Response (SIR).

level of pro-growth factors, less induction & energy supply for LRSC leading to fatigue of LRSC, which then requires another implantation of MSC to increase & maintain the momentum of healing and regeneration [Figure 3]. These apoptotic MSC continue to help in creating pro-environment; their phagocytosis results in reduced responsiveness to lipopolysaccharide (LPS, 58) and preventing macrophage from preventing IL-1 β and TNF- α ; which promotes an anti-inflammatory environment (49, 59). Phagocytosis also affects immune cells that ingest these apoptotic cells leading to the prevention of CD4⁺ T helper cells induction, making the effector lymphocytes ineffective and promoting the clonal expansion of Foxp3⁺ Treg cells (58, 60).

Efficacy & Safety of MSC Therapy**Efficacy of MSC therapy in osteoarthritis of the human knee**

Literature regarding the efficacy of MSC is conflicting and debatable. Most the studies have shown that MSC therapy improves the VAS pain score, WOMAC score & functions compared to placebo or conventional

treatment and it lasts up to 6- 12 months and then it starts showing a downward trend (58, 61-68). While one study reported improvement with both MSC and placebo up to 18 months, and then the placebo group showed a reduced response, but the MSC group continued to perform better than MSC (69). Three studies showed no significant difference between MSC and placebo treatment (70-72). One double randomized study suggested an improvement in knee pain & meniscal volume after one single injection of bone marrow-derived MSC (allogenic) following partial meniscectomy, but it did not reach predetermined significant improvement (15% volume) (73). The author suggested implanted MSC stay on the damaged meniscal surface, differentiate into chondrocytes, and increase extracellular matrix protein and lead the regeneration of the meniscal tissue (73). Literature is also variable and debatable about the potential for density, viability, quality, proliferation, differentiation, and efficacy of MSC from different sources and methods of preparation. Dosage of MSC varies from 5-50 x10⁶ in various clinical trials; some studies report better results with a high dose of intra-articular MSC while

Environment: Pro-environment or Anti-environment depends upon initial pathology and healing response.

- **Anti-environment:** If healing response is less or severe initial damage
- **Pro-environment:** If initial damage is less or good healing response

Mediator: Mesenchymal Stem Cell + Local Resident Stem Cell+ Growth Factors/Cytokines

Mesenchymal Stem Cell:

- **L:** Limited release of growth factors/cytokines/exosomes
- **I: Inhibition:** Apoptosis/Anti-environment
- **R:** Restricted recruitment of new MSC & LRSC

Local Resident Stem cells: Are not so active

- Reduced number (Self-renewal)
- Reduced energy reserves (Exosomes from MSC)
- Reduced secretion of pro-growth factors/cytokines
- Apoptosis

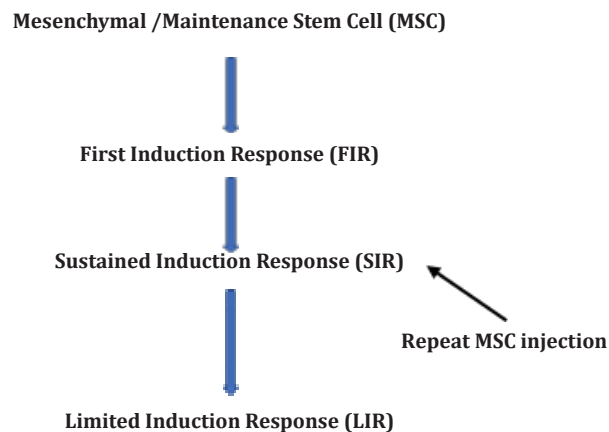


Figure 3. Proposed Mechanism of Mesenchymal/Maintenance Stem Cell (MSC): Limited Induction Response (LIR)

other studies show beneficial effects at low dosages (33, 65, 66, 74). Route of administration included intra-articular, subcutaneous, intra-lesional, intravenously, encapsulated, and integrated administration (33). Many studies have also done x-rays or MRIs to see changes in the cartilage or joint space. X-rays did not show an increase in joint space or improvement in the femoral-tibial angle with the weight-bearing line (64, 74). Three randomized controlled studies report that MSC therapy shows reduced subchondral bone edema, the extension of repair tissue, stabilization of pathology & increased cartilage thickness on MRI at 1-2 years follow up; but these studies used MSC as an adjunct to high tibial osteotomy; while other shows only clinical improvement with no radiological improvement (61-64, 74).

Recently, first long-term follow-up (96 weeks) study done by Song et al. demonstrated the good efficacy of culture-expanded Adipose-Derived Stem Cell (ASCs) therapy in knee OA with multiple injections (75). After showing the safety and efficacy of ADSCs preclinically in vitro and BALB/c-nu nude mice, they did human study. Eighteen patients were divided into three dose groups: the low-dose, mid-dose, and high-dose groups (1×10^7 , 2×10^7 , and 5×10^7 cells, respectively), and were given three injections and were followed up for 96 weeks. They recommended intra-articular injections of MSCs as safe and effective and it improve pain, function, and cartilage volume of the knee joint, with better results in the high dosage group (5×10^7). Spasovski et al. (76) have shown that single intra-

articular injection of ADSCs at a concentration of ($0.5-10 \times 10^7$) in osteoarthritis of knee joint improves clinical symptoms, reduces pain and improves knee score at three months; with maximum benefit usually occurring at six months; without any radiological deterioration (76). Julein Freitage conducted a first randomized study to see the safety & efficacy of culture-expanded ADSCs in 30 patients of osteoarthritis of the knee (77). Patients were randomized into three groups; two treatment groups received either a single injection (100×10^6 ADMSCs) or two injections (100×10^6 ADSCs at baseline and six months); the third group consisted of control and continued conservative management. At 12 months follow-up, a statistical and clinically significant improvement was seen in pain and function in both treatment groups against control and baseline values as measured by validated outcome scores including Numeric Pain Rating Scale, Western Ontario and McMaster Universities Arthritis Index and Knee Injury and Osteoarthritis Outcome Score. Radiographic analysis using the Magnetic Resonance Imaging Osteoarthritis Knee Score indicated modification of disease progression. They recommended ADSCs therapy safe and effective as only minor side effects of pain and swelling were seen in a few patients.

Safety of MSC therapy

Most of the studies report MSC therapy as a safe and effective treatment. Commonly reported less serious adverse effects include post-injection temporary pain

or swelling (up to 50-60% patients), dehydration, and pain due to progressive disease (78, 79). Serious complications, including infection at the iliac crest harvest site, unstable angina, synovial effusion, and pulmonary embolism (67), are infrequent and may not be directly related to MSC treatment (67, 70, 80). Although there may be a theoretical risk of tumor formation with MSC therapy, many studies have rejected this risk or previous claims of tumor formation (68, 72, 79). A standard, safe, and aseptic technique for harvesting, culturing medium, expansion, and quality control will minimize the risk of the complications, as mentioned earlier.

Future Perspectives

How we can improve stem cell therapy

Sites of injection

Currently, intra-articular injection is the most commonly used site of MSC injection. However, since the pathology is not only in the joint space, also most of the implanted MSC cell dies after intra-articular implantation; the author believes that intra-articular injection (suprapatellar pouch) should be supplemented with additional injections in the infra, suprapatellar fat pad and subchondral bone; which might give a more sustained MSC action due to better availability, viability and longer retention of implanted MSC.

Optimum dosage

Although both low (1×10^6) and higher dosage (10×10^7) have been reported to have a beneficial effect, more randomized studies are required to find the optimum dosage & interval with maximum safety.

Delivery of MSC

Implantation of MSC cells in encapsulated form, in the scaffold (with various growth factors; beta transforming

growth factor) and nanoparticle, might perform better by precise target site delivery, with optimum dosages and sustained function of MSC at the implanted site (81).

Maintenance Stem Cells (MSC) may be a more suitable term than mesenchymal stem cell or medicinal signaling cells as their origin might not be limited to mesodermal tissue, they are capable of self-renewal and differentiation into other cell types like osteoblast, adipocyte & chondrocytes

Only a small percentage of implanted MSC survive and rest undergo apoptosis after releasing growth factors, cytokines, and extracellular vesicles. These surviving MSC become active due to confirmational changes induced by anti-environment stimuli and undergo limited self-renewal, proliferation, and differentiation, but only a few of them might incorporate into the host tissues, chondrocytes or chondroblast; these cells generate & maintain a momentum of series of regenerative activities to improve the function of joint, stabilize or possibly enhance the cartilage quality. More randomized studies with long term follow-up are required to bring clarity on their ideal source, expansion, culture technique, optimum dosage and route of administration, and long term safety issues.

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