State of The Art
Hip Knee J. Vol. 3, No. 2, 2022, pp. 56-64
p-ISSN: 2723-7818
e-ISSN: 2723-7826
http://dx.doi.org/10.46355/hipknee.v3i2.133

MESENCHYMAL STEM CELLS SECRETOME AND OSTEOARTHRITIS

Bintang Soetjahjo[1], Dwikora Novembri Utomo[2]
[1] Department of Orthopaedic and Traumatology Dr. Moewardi General Hospital, Universitas Sebelas Maret, Solo
[2] Department of Orthopedic & Traumatology, Dr. Soetomo General Hospital, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

No agreed cure or procedure can prevent the damage caused by OA. Current treatments such as physiotherapy, antiinflammatory drugs, and viscous supplements are symptomatic and are aimed solely at pain relief. Mesenchymal stem cells, on the other hand, refer to a population of cells with immunomodulatory and homing properties. Recent studies have shown that stem cells produce secretomes with tissue regeneration, immunomodulation, anti-inflammatory, and antiapoptotic capacities. Secretomes in conditioned media produced by mesenchymal stem cells can stimulate the repair of cartilage defects. There are many evidence from many studies both in vitro and in vivo which provide potential results. The improvement can be seen from the morphology, in the form of thickening of the cartilage, an increase in the number of chondrocyte cells, regular joint surfaces, and histologically it can be seen that there is an improvement in the joint cartilage matrix. Likewise, histological studies using immunohistochemistry also showed an increase in the expression of TGF-β, SOX-9, aggrecan, and type II collagen which is a pathway for the formation of hyaline cartilage repair. Not only improvement, other studies have also shown that the secretome can provide a protective effect on joint cartilage by increasing the expression of the COL2A1 gene which functions to form collagen matrix components and reduces apoptosis in chondrocyte cells. Secretomes produced by mesenchymal stem cells can stimulate the repair of cartilage damage, anti-inflammatory, immunomodulator, angiogenesis, and anti-apoptotic abilities from their cytokines and extra vesicles contains miRNA. MSCs secretomes are more stable and provide simpler manufacturing than MSCs themselves.

Keywords: Osteoarthritis, Secretome.

Article History:
Submission : August 30th, 2022
Revision : August 30th, 2022
Accepted : August 30th, 2022

Corresponding Author:
Bintang Soetjahjo
Department of Orthopaedic and Traumatology Dr. Moewardi General Hospital, Universitas Sebelas Maret.
Bintangsoetjahjo@gmail.com

INTRODUCTION

Osteoarthritis (OA) is a joint disease that affects synovial joints and can cause damage (1). Osteoarthritis is a chronic, progressive degenerative disease of unknown etiology that is affected by risk factors such as age, obesity, physical activity, and other genetic factors (1). Based on World Health Organization (WHO) data, the global prevalence of OA is 9.6% for men over the age of 60 and 18% for women. In Indonesia, of the total population of 255 million in Indonesia, the prevalence of OA has reached 15.5% (± 39 million) for men and 12.7% (± 32 million) for women (2,3). In addition to the high prevalence of OA, the disease can lead to disability, restrictions on daily activities, and limited movement of joints due to pain, which has significant social and economic implications (2,4). No

agreed cure or procedure can prevent the damage caused by OA. Current treatments such as physiotherapy, anti-inflammatory drugs, and viscous supplements are symptomatic and are aimed solely at pain relief. Other previously performed treatments include bone marrow stimulation (microfracture), osteochondral autologous transplantation (mosaicplasty), and Autologous Chondrocyte Transplantation (ACI). However, these treatments are ineffective because they are difficult, invasive procedures, need donor site availability, morbidity, and fibrocartilage formation (5). In addition, many patients will eventually need to undergo joint replacement after this treatment (5).

Currently, cell-based therapies such as mesenchymal stem cells (MSCs) are attracting the attention of many researchers and clinicians as promising regenerative therapies (6). Mesenchymal stem cells, on the other hand, refer to a population of cells with immunomodulatory and homing properties. However, some researchers have recently argued that MSCs should be renamed "medical signaling cells." Instead of differentiating into tissue-producing cells, these cells secrete therapeutic regenerative bioactive factors that stimulate patient's tissue-specific resident stem cells (7). Recent studies have shown that stem cells produce conditioned media (secretome, extracellular (EV), and exosomes) with tissue regeneration, immunomodulation, anti-inflammatory, and anti-apoptotic capacities (6).

Mesenchymal stem cells secrete a variety of factors, including growth factors, chemokines, cytokines, and hormones. These factors allow stem cells to differentiate in tissue, matrix synthesis, angiogenesis, repair, and immunoregulation (6). The various secretions of these stem cells are in a conditioned medium, in the form of protein compounds, less immunogenic than cells, easier to store, less likely to become tumor cells, and more easily reach target tissues (8). Therefore, it is more stable because it is smaller than cells and current research is shifting from mesenchymal stem cells to conditioned media containing secretomes, paravesicles, and exosomes due to their ability to overcome the limitations of MSCs (8).

However, the number of approved MSCs-based treatment for orthopaedic worldwide

remains limited. In Indonesia, only orthopaedic and plastic surgery department proposed for MSCs-based treatment and has not been approved.

Mesenchymal stem cells (MSCs) also have the ability to renew or regenerate themselves, so that mesenchymal stem cells can make copies of cells that are exactly the same as themselves (9). However, in reality, under normal conditions, in vivo MSCs are mostly in a quiescent phase without differentiation, which contributes to maintaining tissue homeostasis (10). In vivo, MSCs actually have the ability to produce and release anti-inflammatory cytokines, as immunomodulators, and bioactive molecules such as proteins, nucleic acids, and lipids that function to carry out tissue repair (10).

Mesenchymal stem cells are found in various parts of the human body. However, MSCs isolated from the Bone Marrow (BM), Umbilical Cord (UC), adipose tissue, and synovium were confirmed to have stem cell-like properties (11). Selecting suitable site MSCs source for treatment and knowing the advantages and disadvantages of each MSCs sources must be considered (11).

Bone marrow was the first extraction site identified (12,13). Because the safety and efficacy of BM-MSCs have been confirmed by multiple clinical studies, BM-MSCs are the most widely used source of MSCs and are characterized by a very high potential for differentiation (13). However, BM-MSC has some limitations. Most importantly, yield is highly dependent on donor characteristics such as medical condition and age, in addition to the potential for differentiation and repair (14). In addition, collecting BM-MSCs is difficult and inefficient, as only 0.001-0.01% of bone marrow cells are MSCs (13,14).

In 2000, human umbilical cord was recognized as an alternative source of MSCs (15,16). Umbilical cord-derived MSCs (UC-MSCs) exhibit rapid self-renewal and differentiation capabilities that promote tissue repair and regulation of the immune response. In addition, these cells are easily accessible with a painless extraction procedure (16). The rapid growth rate of UCBMSC is about 3-4 times that of Adipose Tissue (AT) MSCs (17,18). Researchers claim that the clinical application of UC-MSCs exceeds the limits of BM-MSCs (18). these stem cells from the umbilical cord did not induce tumorigenesis and were more hypoimmunogenic (19). Moreover, the source of stem cells is the umbilical cord, which is a waste product so that it does not cause disability and morbidity to the donor tissue.

Human Adipocyte tissue-derived MSCs (AT-MSCs) were identified in 2001 as another promising source of MSCs due to their accessibility and abundance, as well as their stronger immunosuppressive effects. Unlike BM-MSC, AT-MSC can be extracted in large, concentrated amounts (about 500 times that of BMMSC) using relatively simple procedures (20). Another advantage of AT-MSCs is that it can be extracted from various parts of the human body. However, ATMSCs extracted from different sites show different characteristics (20).

Synovial-derived MSCs (SM-MSCs) were first described in 2001 (21) Like AT-MSC, SM-MSC can be extracted from a variety of sites with site-specific features, such as the condylar fossa and synovial tissue (21). Interestingly, SM-MSCs have broader proliferative potential, polyphyletic potential, and lower immunogenicity compared to other MSCs (21). Due to the high expression of type II collagen, agrecan, and SRY box transcription factor 9, SMMSC has a higher possibility of cartilage formation than MSCs of other sources, and is often used for cartilage repair and joint treatment (21).

Secretome, (also referred to as stromal cell secretion), is the collective term for the paracrine soluble factors produced by stem cells and used for communication between their cells. Apart from intercellular communication, paracrine factors are also responsible for tissue development, homeostasis and regeneration. Stem cell secretomes consist of extracellular vesicles, exosomes, microvesicles, membrane particles, peptides and proteins (cytokines) (5). Extra vesicles are vesicles originating from cells that are numerous and may be present in all fluids including blood, urine, and cell culture media (22). Extra vesicles play a role in intercellular communication and can transport mRNA, miRNA, and proteins between cells (23).

The regenerative and repair abilities of mesenchymal stem cell conditioned media include the presence of growth factors and cytokines in it (24). Another study also showed the advantages of this mesenchymal stem cell conditioned media therapy compared to therapy using mesenchymal stem cells themselves, namely in terms of manufacturing, easier to manage, store, distribute, and easier to administer in allogenic because it does not contain cells in it, while retaining the same superiority as mesenchymal stem cells (8). Comparison between MSCs and MSC secretome can be seen in (Table 1.).

Table 1. Advantages and disadvantages of MSCs and MSCs secretome

Mesenchymal stem cells	**EV & Exosomes**
Advantages	**Advantages**
Low immunogenicity due to absence of HLA-class III antigen, makes them ideal candidate for allogenic transpaltion.	Higher stability
MSCs regenerative and repair mechanisms in their potential to replace damaged cell.	Lower immunogenicity
MSCs are the most common and widely investigated cell type used amongst other cell based therapy.	Longer period of circulation in the body
	Easier storage
	Ability to cross the blood-brain berrier
	Safety for delivery to the target cell due to its adhesion molecules present on their surface
Disadvantages	**Disadventages**
High cost maintance	Promote endogenous repair using resident cell
Long term homing potential	Clustering after freeze-thaw cycles
Transient survival after transplantation	Need to be freeze dried to maontain preservation
MSCs may undergo autophgy and apoptosis to release growth factors and cytokines rich exosomes in turn to alleviate disease pathology	Clumping of exosome and degradation during freeze-drying process
Difficult to store and reuse after revival	

Secretomes in conditioned media produced by mesenchymal stem cells can stimulate the repair of cartilage defects (6).

There are many evidence from many studies both in vitro and in vivo which provide potential results. The improvement can be seen from the morphology, in the form of thickening of the cartilage, an increase in the number of chondrocyte cells, regular joint surfaces, and histologically it can be seen that there is an improvement in the joint cartilage matrix (25–27).

Likewise, histological studies using immunohistochemistry also showed an increase in the expression of TGF-β, SOX-9, aggrecan, and type II collagen which is a pathway for the formation of hyaline cartilage repair (26). Not only improvement, other studies have also shown that the secretome can provide a protective effect on joint cartilage by increasing the expression of the COL2A1 gene which functions to form collagen matrix components and reduces apoptosis in chondrocyte cells (27).

MSC secretomes were found to be different from other secretome. The differences consist of the presence of angiogenic factors, lower amount of Metalloproteinases (MMPs), and high production of TGF-β, chemokines and anti-inflammatory cytokinesthat make the UC-MSC secretome a perfect source to control inflammation (8). Col II and Aggrecan were produced to maintain extracellular matrix homeostasis (27). There are many cytokines considered to be the source of paracrine signal as an immunomodulator, anti-inflammation, angiogenic, and cartilage repair.

However, only some cytokines have been identified. More specifically, thrombospondin-2, a glycoprotein mediating cell to cell and cell to matrix interactions is responsible to activate signalling pathways in chondrogenesis and cartilage regeneration via ERK and p38/MAPK signalling pathways (8,28). Thrombospondin-1 also found in UC-MSC secretome which can regulate activation of TGF-β/Smad pathway in chondrocytes for cartilage regeneration process (8).
As an anti-inflammatory effect, there is prostaglandin E2 as an alternate the activation of macrophages, hinder maturation of dendritic cell, and reduce cytotoxicity of NK cell (8). IL-10 also acts as anti-inflammatory cytokine to reduce TNF-α, IL-1β, IL-6 (5).

Besides cytokines, secretome also contain extra vesicles (EVs) with RNA KLF3-AS1 that can inhibit miR-206 which can cause progression of OA and promotes senescence (overexpressed in OA) (8). EVs also contain miR-21 as the anti inflammatory effect to down regulate TNF-α-induced apoptosis (5).

Moreover, to stop the progression of OA, miR-92a-3p contained EVs can act as Wnt inhibitor and suppress expression of ADAMTS4 and ADAMTS5 to delay progression of OA (5). As a regeneration process, there is miR-140-5p contained EVs to promote chondrocyte proliferation and migration and inhibit chondrocyte hypertrophy via the Wnt5a/NFkB and Wnt5b/JNK pathways (5). The main role of microRNAs in EVs in pathogenesis of OA is summarised in (Table 1.) (29). At present, most researcher prefer to obtain the

secretomes for OA therapy with high expression of miRNA or lncRNA from MSCs which have been reported to have significant effects on OA in vivo and in vitro (30).

Some clinical trials that used MSC treatments in patients with OA. Kim et al used AT-MSCs loaded into fibrin glue and implanted via arthroscopic procedure in 49 patients (31). In this study, patients showed overall satisfaction with improved mean IKDC and Tegner activity score (31). In Shapiro et al study, used BM-MSCs combined with platelet-poor plasma and injected to 25 patients' knees (32).

Significant improvements in ICOAP scores, VAS pain scores, and activity level (32). Matas et al used UC-MSCs as intraarticular injection to 26 patients' knee joints (33). Improvement in pain and function with lower WOMAC and VAS pain scores were reported (33). There were no serious adverse events were reported. Other clinical trials using ATMSCs from Pers et al, Freitag et al, and Lee et al also showed improvement in pain and functional level (34–36).

In vitro study of UC-MSCs secretome for therapy of arthritis have been searched. Saulnier et al., using equine UC-MSCs secretome to treat OA in rabbit IL-1β synoviocytes as target cells showed decreasing in MMP-1, MMP-3, MMP-13, IL1β, and TIMP which can lead to stop the progression of OA process. Widowati et al., targeting human chondrocyte cell line + IL-1β showed increasing IGF-1 for chondrogenesis process and decreasing ADAMTS1, MMP-1, and MMP-3 to stop pathological process of OA. Wang et al., and Chang et al., also targeting human chondrocytes and showed increasing proliferation of MSC, increasing cell viability, aggrecan, SOX-9, collagen type II, and decreasing inflammatory factors and apoptosis.

In vivo studies also showed the improvement of cartilage repair in 28 studies after application of injection secretome in OA animal models (5). The cartilage repair process using secretome injection in OA models showed increasing in TGF-β, SOX-9, aggrecan, and collagen type II which are the hallmarks pathway of cartilage repair and regeneration (25,26). However, the clinical studies of MSCs secretome to treat OA patients are still needed and on going.

CONCLUSION

Secretomes produced by umbilical cord mesenchymal stem cells can stimulate the repair of cartilage damage, anti-inflammatory, immunomodulator, angiogenesis, and antiapoptotic abilities from their cytokines and extra vesicles containing miRNA. UC-MSC secretomes are more stable and provide simpler manufacturing than UC-MSCs themselves. They are also rich in cytokines and miRNA that can be a potential therapy for OA in the future.

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