Chapter 3

Stem Cells for Orthopaedics Application

Ismail Hadisoebroto Dilogo

A. Introduction

Over the past decade, fundamental science and experimental studies concerning stem cells have expanded. The use of stem cells in orthopaedics is expanding in line with the rise of basic scientific research. Stem cells in orthopaedic cases are transforming orthopaedic practices towards regenerative medicine, beginning with the treatment of bone abnormalities and the regeneration of nerves, tendons, ligaments, and cartilage (Maniar et al., 2015). Stem cells are cells with the ability to divide, self-renew, and differentiate into several types of body cells. On the basis of their differentiation potential, stem cells are classified as totipotent, pluripotent, or multipotent. Totipotent stem cells are capable of differentiating into any type of human cell, including placental and extraembryonic cells. Only embryonic stem

I. H. Dilogo

Universitas Indonesia, e-mail: ismailortho@gmail.com

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cells can produce totipotent stem cells. However, the use of totipotent stem cells in clinical practice has not been established due to medical ethics problems. Endoderm, mesoderm, and ectoderm are the three embryonic layers that pluripotent cells can transform into. Precisely, pluripotent stem cells originate from embryonic cells after the blastocyst phase. Multipotent stem cells, unlike their predecessors, can differentiate into more than one germ layer, but not all three. Adult stem cells and blood stem cells are examples of mesenchymal stem cells (MSCs). There are bones, cartilage, muscles, and ligaments involved in orthopaedics. MSCs have the ability to differentiate into mesoderm-derived tissues. Therefore, the use of mesenchymal stem cells is one of the most common in orthopaedic disorders (Im, 2017).

According to study done by Kabat et al. in 2018, the bulk of clinical studies employing MSCs were undertaken by the Neurology Department. In clinical studies involving mesenchymal stem cells, orthopaedics comes in second place. The concept of tissue engineering, especially in orthopaedics, adheres to the 5 R principles: repair, replace, restore, regenerate, and rejuvenate. MSCs can be either autologous or allogeneic, with its own advantages and disadvantages. Allogenic MSCs sources have advantages, including being more ready to use and not causing donor-site morbidity. Allogenic MSCs have also been proven safe to use in clinical trials since it has low HLA-II expression and triggers minimal immune reactions. In addition, allogeneic MSCs also do not trigger lymphocyte proliferation and modulate the immune system. The bulk of clinical studies employing MSCs were undertaken by the Neurology Department, according to study published in 2018 by Kabat et al. In terms of clinical studies utilizing MSCs, orthopaedics is ranked second (Gerth et al., 2019).

B. Stem Cells for Critical-Sized Bone Defects

Fracture is one of the most common cases encountered in orthopaedics and traumatology. Unlike other organs, bones can heal perfectly as before a fracture occurs (Santolini et al., 2015). The fracture healing process is depended on the diamond concept, which Giannoudis first

introduced. The diamond concept states that for a fracture to achieve optimal healing, four pillars must be addressed: osteogenic cells, osteoconductive, osteoinductive, and mechanical stability (Giannoudis et al., 2007). The osteogenic column is comprised of osteoprogenitor cells from the periosteum and endogenous MSCs from the bone marrow. Shortly after the fracture, the creation of a hematoma in the region around the fracture activates these cells. The production of cytokines initiates an inflammatory phase marked by increased vascularity and permeability of the blood vessels. Osteoclasts and fibroblasts transform the hematoma into granulation tissue and lay down a fibrin meshwork, which is eventually penetrated by vascular capillaries after the migration of MSCs. Endothelial cells, MSCs, chondrocytes, osteocytes, and osteoblasts then produce cytokines. This procedure is followed by the proliferation and differentiation of MSCs to create hard and soft calluses (Schubert et al., 2013).

Osteoconductive pillars are scaffolds, specifically the extracellular matrix, which works as a scaffold and supports the migration and adhesion of osteoinductive and osteogenic cells at the fracture site, which is necessary for fracture healing. In fractures when there is insufficient scaffolding, an autograft or allograft is necessary. Previous research has revealed that cells can perceive the mechanical environment surrounding them through electrochemical signals created by fluid changes in the canaliculi. Different cell membranes also serve as mechanoreceptors in a variety of other cell types (Utomo et al., 2019; Shang et al., 2021).

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A critical-sized bone defect is defined as one that is greater than 2.5 times the bone's diameter. The primary issue with significant bone abnormalities is the restricted capacity of the fracture and its surrounding environment to mend optimally. In the initial stages of the healing process, the hematoma serves as a source of signaling chemicals that stimulate cellular cascades. MSCs are drawn to the fracture site and develop into osteoblasts in response to growth factor stimulation to enhance fracture repair. In MSCs-based treatment, the migration of MSCs to the wounded site is considered the initial stage in bone production and defect healing. BMPs and platelet-derived growth factors (PDGFs) are crucial for bone production and fracture repair, while MSCs stimulate angiogenesis to promote bone regeneration (Dilogo et al., 2017; 2019).

Ten to fifteen percent of the time, the healing and regeneration of bones, particularly those with substantial abnormalities, are disrupted, resulting in delayed union and non-union. Non-union has several reasons, including bleeding problems, periosteum injury, bone loss, and poor fracture stabilization. The gold standard for stability is the administration of an autologous bone transplant. However, this approach is limited by its limited graft resources. Despite the effectiveness of autologous bone grafting surgeries, problems such as prolonged discomfort at the donor site (49%), deformity, and impaired function still occur. The utilization of autologous or allogenic MSCs is crucial for the development of cell-based therapies for nonunion patients (Giannoudis et al., 2016).

After the inflammatory phase, the wounded region recruits endogenous MSCs, which then develop into chondrocytes or osteoblasts. Chondrocytes undergo bone mineralization by endochondral ossification, whereas osteoblasts deposit bone via intramembranous ossification. Periosteum, endosteum, and bone marrow are sources of endogenous MSCs that can promote bone repair. Endogenous stem cells release a variety of bioactive molecules that influence tissue function and possess anti-inflammatory, immunomodulatory, and regenerative capabilities. In addition to endogenous stem cells, foreign

stem cells can also be injected. Exogenous MSCs acts as an osteogenic, osteoinductive, and osteoconductive function in promoting fracture repair (Knight & Hankenson, 2013). MSCs factor can be administered systemically via the circulation, or it can be administered locally at the fracture site. Our previous studies have shown that MSCs are still found in areas with atrophic non-union fractures and can differentiate into osteogenic cells (Ismail et al., 2013).

The concept of tissue engineering in orthopaedic cases saves time, reduces complications and lengthy surgical procedures, reduces donor site morbidity, and provides effective results. After the inflammatory phase, endogenous mesenchymal stem cells are recruited and differentiated into chondrocytes or osteoblasts in the damaged region. Osteoblasts deposit bone by intramembranous ossification, whereas chondrocytes mineralize bone via endochondral ossification. The periosteum, endosteum, and bone marrow are sources of endogenous MSCs that can promote bone repair. Endogenous stem cells release many bioactive substances that influence tissue function and possess anti-inflammatory, immunomodulatory, and regenerative capabilities. Exogenous stem cells can be supplied in addition to endogenous stem cells. In clinical use, BMP (Bone Morphogenic Protein) also has some side effects. These side effects include leakage and triggering ectopic bone formation. In addition, BMP-2 increases osteoclastic activity, thereby increasing osteolysis. BMP also induces local inflammation and forms a seroma or soft tissue swelling (James et al., 2016). BMP-2 is the only FDA-approved osteoinductive growth factor for clinical usage at a 1.5 mg/ml dosage (Kamal et al., 2019; Pearson et al., 2019).

Six patients with critical-sized bone lesions were treated with a combination of Bone Marrow MSCs (BM-MSCs), hydroxyapatite (HA) granules, BMP-2, and internal fixation in a translational trial. Within six months of follow-up, we discovered that the patient's pain rating decreased dramatically. Within one year of follow-up, clinical and radiological results were also dramatically improved, and no adverse effects, such as tumorigenesis, were noted (Dilogo et al., 2019). In addition, we conducted a trial employing umbilical

cord mesenchymal stem cells (UC-MSCs) to treat seven patients with critical-sized bone lesions. In addition, we discovered that all patients reported considerable functional improvement during a follow-up period spanning from 12 to 36 months (Dilogo et al., 2021).

C. Stem Cells for Articular Cartilage Defect

Articular cartilage consists of an extracellular matrix and chondrocytes that absorb and even out the mechanical loading received by the joint. However, articular cartilage has poor vascularization and does not have good healing potential. Damage to the cartilage that is sustainable will develop into end-stage arthritis. Various surgical modalities are known to treat articular cartilage defects, such as the microfracture method, mosaicplasty, and osteochondral grafting. However, the outcome of surgical modalities such as microfracture and osteochondral grafting will result in cartilage repair in the form of fibrous cartilage. Studies have also shown a tendency towards failure with surgical modalities. Therefore, recent research has moved towards stem cell-based therapy to slow or reverse cartilage damage (Dewan et al., 2014; Phull et al., 2016).

Mesenchymal stem cells can be grown culturally to form various tissues derived from the mesoderm, including cartilage. In the defect area, MSCs release bioactive factors, which are regenerative and immunomodulatory. Factors secreted by exogenous MSCs stimulate endogenous MSCs in the body and form new cartilage tissue. Endogenous MSCs are found in many synovial joint tissues, representing 1% of the total cell population. It modulates the inflammatory cascade, which is essential in cartilage repair. Unfortunately, endogenous MSCs decline functionally with age (Lam et al., 2020; Le et al., 2020; Lee et al., 2021).

Chondrogenesis begins with the condensation of progenitor cells, which is then followed by the migration of MSCs. This process leads to the creation of cartilage and bone via endochondral ossification. To repair cartilage injury, cartilage damage will activate the creation of chondrogenic factors that recruit endogenous MSCs

from the synovium, synovial fluid, and bone marrow. The MSCs and scaffold are then placed at the fault site. Scaffolds offer MSCs with a microenvironment in three dimensions for proliferation and differentiation. Endogenous MSCs that have been stimulated will release bioactive substances that alter tissue function and have anti-inflammatory, immunomodulatory, and regenerative effects (Xiang et al., 2022; Zha, Li, et al., 2021; Zha, Sun, et al., 2021).

Exogenous sources of MSCs can be administered by direct implantation through a surgical incision for cases of severe defects or by intra-articular injection for small defects. If not satisfied with MSCs administration, we can add chondrogenic factors and scaffolds to increase cartilage repair. Chondrogenic factors include bone morphogenic protein (BMP), transforming growth factor beta (TGF-B), insulin-like growth factor-1 (IGF), and platelet-rich plasma (PRP). BMPs, particularly BMP-2 and BMP-7, promote cartilage regeneration by promoting MSCs differentiation and increasing the recruitment of endogenous MSCs to injured cartilage regions. Chondrocytes are effectively stimulated to produce proteoglycans and type 2 collagen by transforming growth factor-beta. IGF-1 is a cartilage homeostasis mediator with the function of increasing chondrocyte proliferation and stimulating proteoglycans. PRP also stimulates the proliferation of MSCs and enhances the formation of extracellular matrix (Goldberg et al., 2017).

A scaffold is required and must be present for cartilage regeneration. To be regarded suitable for cartilage regeneration, scaffolds must satisfy three requirements. These criteria include being composed of biodegradable and biocompatible materials to support the chondrogenesis process, having a degradation rate that adapts to the rate of cartilage formation, and possessing mechanical properties that can withstand physical loading to provide adequate space for the tissue to undergo the regeneration process (Daneshmandi et al., 2020; Kadir et al., 2021; Muhammad et al., 2019).

MSCs activities in tissue regeneration include chondrocytes, extracellular matrix, the immune system, mitochondria, and paracrine

actions. Against chondrocytes, MSCs enhance chondrogenesis, decreases apoptosis, and preserves chondrocyte autophagy. MSCs balance the ratio of MMP-13 to TIMP-1 in cartilage and decreases the expression of hypertrophy indicators in cartilage, including collagen X, FGF receptor, PTH-related protein, and MMP-13. MSCs promote immunosuppressive factors and hinders the maturation of monocytes into dendritic cells. Through mitochondrial transfer, MSCs enhance the membrane potential in mitochondria. MSCs have paracrine actions that are mediated via extracellular vesicles produced from MSCs (Platas et al., 2013; Stoddart et al., 2015).

Supartono et al. found in a prior study that microfractures had no meaningful effect on profound osteochondral abnormalities. Therefore, the inflammatory process without microfractures is adequate to drive MSCs homing (Supartono et al., 2018). A systematic review by Dilogo et al. demonstrated that intra-articular umbilical cord MSCs injection without surgical treatment improved clinical outcomes in patients with knee osteoarthritis. This finding is supported by the ability of MSCs to reduce cartilage erosion due to synovitis in pre-clinical studies without any reports of serious adverse events (Dilogo et al., 2023).

During cartilage development, MSCs contribute to the production of an extracellular matrix that is crucial for cartilage repair. In addition, MSCs produce cytokines, growth factors, and chemokines that recruit endogenous MSCs to the site of the defect and maintain the optimal microenvironment. Despite all the benefits of MSCs, their use has a number of drawbacks, including the inability to be used immediately and high storage costs. Based on the limits of MSCs, research has also demonstrated that the therapeutic potential of MSCs derives from their paracrine components, which are also present in their derivative product, the secretome. The secretome, also known as conditioned medium (CM), is a substance that is released by MSCs and comprises extracellular vesicles and other biological components, such as growth factors and cytokines. Due to its cell-free nature, secretome offers a lesser risk of immunogenicity compared to MSCs, as well as a manipulable dosage and potency, large-scale production, ready-to-use

status, comparatively cheap cost, and storage simplicity. Secretome's therapeutic impact may be attributed to its pro-angiogenic, anti-fibrotic, anti-apoptotic, anti-inflammatory, and immunomodulatory properties. In contrast to MSCs, however, secretome has limitations such as a shorter half-life that necessitates repeated administration and a poorer anti-inflammatory capacity. The secretome increases collagen type 2 expression to preserve cartilage integrity and viability. It enhances matrix synthesis by inhibiting the generation of nitric oxide. Additionally, the secretome lowers chondrocyte inflammation by downregulating degradation enzymes and pro-inflammatory cytokines (such as Interleukin 6 and TNF alpha) and upregulating anti-inflammatory cytokines such as interleukin 10 (Chang et al., 2018; Contentin et al., 2022).

Due to its immunomodulatory, regenerative, anti-catabolic, and chondroprotective capabilities, secretome administration has been shown to be useful in instances of osteoarthritis by preclinical research. Previous research by Lubis, Luthfi et al. stated that secretome administration from UC-MSCs, along with microfracture procedures, is effective as an alternative treatment for cases of cartilage defects (Lubis, Luthfi, et al., 2022). Growth hormone (GH) is a biological factor that has the potential to regenerate cartilage by regulating the body's metabolism, namely by talking about the production of insulin-like growth factor (IGF-1). However, during the ageing process, there is a phenomenon known as somatopause, so giving external GH becomes rational. Giving GH and regulating IGF-1 also triggers neovascularization, which is crucial in morphoangiogenesis. In addition, GH also stimulates chondrocytes to synthesize matrix components such as type II collagen, which is essential in cartilage regeneration. Lubis et al. (2019) also shown that the treatment of intra-articular growth hormone injections resulted in improved macroscopic and microscopic results in osteoarthritis in New Zealand rabbits than the administration of hyaluronic acid and no injection (control group). In subsequent studies, Lubis, Wijaya, et al. demonstrated that repeated treatment of growth hormone had a

greater effect than a single dosage, specifically by administering five injections at 1-week intervals. Lubis, Wijaya, et al. also reported in their comprehensive analysis that growth hormone injections had encouraging outcomes for cartilage regeneration in knee osteoarthritis without systemic side effects (Lubis, Wijaya, et al., 2022).

The development of stem cells and their derivatives in cartilage regeneration is growing. Currently, exosome research, especially in pre-clinical studies, is increasingly supporting their use in cases of cartilage regeneration. Exosomes are extracellular vesicles measuring 50–130 nanometers which function for intercellular communication and signal transduction between cells. The advantages of using exosomes include having a potent paracrine function, less rejection from the immune system, no risk of triggering tumors, can be combined with existing carriers, and being easier to store (Contentin et al., 2022; Lee et al., 2021). Exosomes induce endogenous stem cells, increase endogenous chondrocytes, and decrease pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha to regenerate cartilage via many methods. Exosomes were shown to boost cell proliferation and minimize the frequency of apoptosis in an in vitro examination of osteoarthritis patients in the temporomandibular joint. Exosomes from Bone Marrow MSCs successfully enhance cartilage repair and extracellular matrix formation and relieve pain in rabbits with knee osteoarthritis, according to additional preclinical research (Lee et al., 2021; Liu et al., 2019).

D. Stem Cells for Avascular Necrosis of Femoral Head

Exosomes contain many pathways for cartilage regeneration, including activating endogenous stem cells, boosting endogenous chondrocytes, and suppressing pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha. In an in vitro examination of osteoarthritis patients in the temporomandibular joint, exosomes were shown to boost proliferation and decrease apoptosis incidence. Exosomes derived from bone marrow mesenchymal stem cells efficiently stimulate cartilage repair, extracellular matrix formation, and pain reduction

in rabbits with knee osteoarthritis, according to another preclinical research (Xu et al., 2020).

Early therapy results in a favourable clinical outcome. Unfortunately, many patients present with severe osteonecrosis and missed opportunities in the early stages of development, necessitating THR (Total Hip Replacement). THR has downsides, particularly in young adult patients, because of decreased mobility and the need for revision. Osteonecrosis of the femoral head is an orthopaedic disorder characterized by disruption of blood vessels and necrosis of the subchondral bone, leading to femoral head necrosis. In addition, this illness is defined by elevated intra-osseous pressure and disturbances in bone metabolism, which result in an imbalance between bone absorption and remodeling. Now available therapies include core decompression, vascularized bone graft, osteotomy, tissue engineering material transplantation, and complete hip replacement. No medicine, however, can halt the progression of avascular necrosis. Forty percent or more of patients who had core decompression required total hip arthroplasty due to disease progression (Houdek et al., 2014).

There are several approaches to utilizing stem cells in femoral head avascular necrosis cases, including combining stem cells with core decompression, autologous bone transplant, platelet-rich plasma, and other biomaterials. Other research suggests that using stem cells can improve short-term therapeutic outcomes. Mao et al.'s recent meta-analysis indicated that stem cell treatment could dramatically decrease development and increase the long-term survival of the hip joint. Furthermore, the study by Mao et al. explained that the administration of stem cells was most effective, particularly when given to individuals under the age of 40 (Mao et al., 2020).

The most prevalent treatment for femoral head osteonecrosis is MSCs therapy. Depending on the source, MSCs can develop from bone marrow, adipose tissue, peripheral blood, or the umbilical cord. Unknown is the precise manner of stem cell-based treatment for avascular necrosis of the femoral head. One rationale is the biological characteristics theory, which asserts that stem cells may self-replicate

and replenish. When stem cells are injected into a necrotic femoral head, they can develop into osteoblasts, chondrocytes, and other tissues, enabling the regeneration of dead bone. In addition, stem cells release numerous biological substances like cytokines, growth factors, and exosomes to promote angiogenesis, reduce femoral head hemorrhage, and reduce intra-osseous pressure, therefore decreasing the course of femoral head osteonecrosis (Li et al., 2021).

Houdek et al.'s paper discusses the indications and contraindications for using stem cells in femoral head avascular necrosis cases. According to the Steinberg classification, additional indications include individuals with symptomatic stage 1 or stage 2 avascular necrosis of the femoral head. On MRI, patients show bilateral symptomatic avascular necrosis on at least one side and >30% asymptomatic lesions. Stem cell administration is contraindicated for patients with avascular necrosis of the femoral head stage 3 or higher, fast-developing avascular necrosis, and active or persistent infections (Elgaz et al., 2020; Houdek et al., 2014).

E. Stem Cells for Brachial Plexus Injury

Brachial plexus injury (BPI) is a damage to the peripheral nerves that results in paralysis of the upper extremities. It occurs in 2.8% of trauma patients. These injuries are frequently the result of high-energy trauma, such as automobile collisions. Injury to the BPI can vary from nerve compression to total nerve transection. A nerve damage is characterized by muscular weakness, altered reflexes, and loss of sensory function. Improving nerve function is hampered by the sluggish regeneration of nerves, which is around 1 mm every day. Various attempts have been made to repair peripheral nerve damage, such as the administration of drugs and also microsurgery interventions in the last few decades. However, there are still limitations regarding functional outcomes in long-term follow-up. The denervation from the injury causes muscle contractility and atrophy loss. A state of denervation left for a long time causes irreversible atrophy. Resident muscle stem cells enable regeneration of skeletal muscle tissue. In muscles that replace injured

myofiber, the regeneration phase is marked by the activation of a population of stem cells known as satellite cells.

To treat motor neuron degeneration, cell replacement procedures have been established throughout the past decade. Due to their capacity to develop into neural progenitor cells, mesenchymal stem cells have garnered attention as a nerve regeneration technique (Sumarwoto et al., 2022). MSCs can also develop into neuronal cells, such as Schwann cells, which aid in nerve regeneration by creating myelin. Mesenchymal stem cells can also stimulate myogenesis and angiogenesis by secreting angiogenic, mitogenic, and anti-apoptotic substances such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and Bcl-2. In addition, MSCs generate paracrine proteins such as stem cell factor and heat-shock protein 20, which enhance organ function by promoting remodeling, regeneration, and neovascularization. MSCs can also move to sites of damage or hypoxia, where they enhance tissue healing. This is due to the release of anti-inflammatory, anti-apoptotic, and trophic substances, such as brain-derived neurotrophic factors and nerve growth factors.

MSCs can be administered locally or systemically. Local MSCs implantation has the advantage that MSCs can directly reach the target organ, also known as "non-systemic homing". Meanwhile, intravenous MSCs implantation has a weakness where MSCs can be trapped in the lungs, liver, or spleen, considering that these organs are more significant, and their adhesion molecules will reduce the number of cells that reach the target site (about 2%). Following the principles of tissue engineering, stem cell implantation can be optimized by creating a micro-environment that supports regeneration by combining MSCs administration with scaffold biomaterials and growth-promoting factors (Guo et al., 2020).

F. Stem Cells for Degenerative Disc Disease

Additionally, MSCs can develop into neuronal cells such as Schwann cells, which promote nerve regeneration by creating

myelin. Mesenchymal stem cells can also stimulate myogenesis and angiogenesis by secreting many angiogenic, mitogenic, and anti-apoptotic substances, including vascular endothelial growth factor (VEGF), IGF-1, HGF, and Bcl-2. In addition, MSCs generate paracrine proteins such as stem cell factor and heat-shock protein 20, which contribute to remodeling, regeneration, and neovascularization, hence boosting organ function. MSCs are also capable of migrating to sites of damage or hypoxia and enhancing tissue healing. This can be explained by the release of anti-inflammatory, anti-apoptotic, and trophic substances in the form of nerve growth factors and brain-derived neurotrophic factors (Xie et al., 2021).

Both clinicians and scientists have been interested in furthering stem cell research in cases of degenerative disc degeneration in recent years. It has been demonstrated that stem cells can postpone or even reverse the process of intervertebral disc degeneration. The deterioration of the discs in the spine causes degenerative disc disease, which leads to inflammation and the production of enzymes that further degrade the tissue. MSCs can aid in tissue repair by differentiating into the necessary repair cells and producing cytokines and growth factors that reduce inflammation and promote healing.

Regarding disc degeneration, stem cells serve three fundamental functions. First, stem cells can differentiate into cells resembling intervertebral discs. The regenerative capability of stem cells implanted in IVD consists of their ability to differentiate into nucleus pulposus cells and stimulate the production of new extracellular matrix. Despite the fact that many studies indicate that stem cells die shortly after implantation owing to a lack of nutrition and pH fluctuations, other studies indicate that MSCs and their progenitor cells can survive when injected in large quantities. By secreting chemokines, components, growth factors, and anti-inflammatory mediators via their paracrine pathways, stem cells can boost the viability of resident cells at their implantation sites. Thus, as extracellular matrix secretion rises, the expression of proteins linked with cell senescence falls. Additionally, stem cells can modify the mechanical characteristics of the nucleus

pulposus and lessen the stiffness of the cell and matrix to increase cell survival. Stem cells can postpone the progression of intervertebral disc degeneration by reducing the control of the immune system. Damage to the extracellular matrix is exacerbated in the presence of pro-inflammatory cytokines that mediate inflammatory processes with interleukins, TNF, interferons, prostaglandin E2, and other chemokines. These pathways promote apoptosis, senescence, and autophagy in cells. Stem cells implanted in intervertebral discs can create anti-metabolic mediators, growth factors, and anti-inflammatory cytokines, according to studies (Zhang et al., 2022).

G. Stem Cells for Ligament and Tendon Healing

As the popularity of amateur and professional sports increases, so does the prevalence of injuries. Both conservative therapy and surgical excision and repair have been used to treat tendon injuries. Traditional treatments such as rest, cold, compression, elevation, a brief course of a pain modulator, and anti-inflammatory medications can reduce pain, but they do not result in total healing. The healing process is extremely delayed because of the inadequate vascularization of tendons and ligaments. Healing cannot restore tendon function due to the production of mechanically weaker scar tissue, the danger of ectopic bone formation, and the limited regeneration of fibrocartilage at tendon-to-bone contact (Trebinjac & Gharairi, 2020).

Due to the limits of tendons' self-repair capacities and existing therapies, it is crucial to have alternative tendon regeneration techniques. Similar to mesenchymal stem cells, tendons and ligaments contain a small number of stem cells known as tendon stem/progenitor cells (TSPCs). TSPCs exhibit all of the features of mesenchymal stem cells, including surface markers, the capability for self-replication, and the ability to differentiate into bone, cartilage, and fat. When transplanted, TSPCs create ectopic tissue. The regeneration of ligaments and tendons has been investigated using embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells. The most study has been

conducted on mesenchymal stem cells, and the results are positive. MSCs can develop into mesoderm-derived tissues such as ligaments and tendons. MSCs are capable of regenerating tendons due to their ability to differentiate into tenocytes, the cells that compose tendon tissue. In addition, MSCs have a high capacity for proliferation and synthesis, which expedites tissue healing. When MSCs are implanted into injured tendons, they differentiate into tenocytes and generate extracellular matrix, aiding in tissue regeneration. In addition, MSCs possess immunomodulatory properties that are beneficial for reducing tendon inflammation (Lui, 2015).

When injected into torn tendons or ligaments, MSCs can release cytokines and growth factors that encourage the body's natural healing response and recruit necessary cells for tissue regeneration. More study has been undertaken on MSCs for tendon repair than on ligaments. In a prior study, Wang et al. compared the injection of 75 million allogenic mesenchymal precursor cells (MPCs) with hyaluronic acid to the administration of hyaluronic acid in post-ACL reconstruction patients. Patients treated with MSCs reported quicker pain alleviation and functional benefits. Injecting MSCs into the partly torn patellar tendon of rats enhanced tendon repair and increased the mechanical strength of the tissue, according to Yin et al. (Yin et al., 2016).

Centeno et al. reported 29 patients with clinical symptoms and MRI confirmation of grade 1-3 ACL tears who were treated with injections of 2-5 cc of bone marrow concentrate derived from 60–120 cc of whole blood marrow aspirate and combined with platelet rich plasma (PRP) and platelet lysate (PL). At 8.8 months follow-up, 77% of patients demonstrated substantially improved ACL integrity, as measured by T1 MRI ACL signal intensity, according to this study (Lui, 2015). A systematic review of the use of a secretome in tendon and ligament healing by Rhatomy et al. found that an MSCs secretome could enhance tendon and ligament healing in preclinical studies. The paracrine effect of MSCs influence its therapeutic effect on tendon and ligament regeneration. The use of stem cell CM as a treatment for tendon or ligament damage could give an alternative to direct stem

cell therapy that is non-invasive and less complicated. Further research is necessary to comprehend the efficacy and safety of stem cell CM treatment in clinical situations (Rhatomy et al., 2020).

H. Conclusion

Stem cells have demonstrated considerable potential in orthopaedics and trauma surgery for initiating healing processes, compensating for deficiencies, and stimulating the regeneration of tendons, muscles, bones, and cartilage. Stem cell therapy has various advantages, including reduced pain, better function and mobility, and tissue regeneration. The success rate of stem cells for orthopaedic disorders is encouraging. However, its limitation including expensive cost, lack of standardization, and limited availability still have to be solved before its usage can be generalized. Therefore, we suggest further research, develop standardized protocols, combining its approach with tissue engineering, and further explore the stem cell-derived metabolites, such as secretomes and exosomes.

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