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# Stem Cell Injections for Enhancing Anterior Cruciate Ligament (ACL) Healing After Graft Repair: A Systematic Review and Meta-analysis

**Gede Agung Dhimasena Widyananda[1], I Gusti Ngurah Wien Aryana[2], I Gusti Ngurah Yuda Bagus Aryana[1], Made Ramanda Bramasta Pramana[3]**

[1]Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

[2]Sport Consultant of Department of Orthopaedics and Traumatology, Faculty of Medicine, Udayana University, Prof. Dr. I.G.N.G. Ngoerah General Hospital, Denpasar, Bali, Indonesia

[3]Resident of Department of Orthopaedics and Traumatology, Faculty of Medicine, Udayana University, Prof. Dr. I.G.N.G. Ngoerah General Hospital, Denpasar, Bali, Indonesia

E-mail: quartalvoicings@gmail.com; dyudaaryana32@gmail.com; Wienaryanaortho@gmail.com; framandabramasta27@gmail.com

*Corresponding author details: Gede Agung Dhimasena Widyananda; quartalvoicings@gmail.com

**ABSTRACT**

***Background:*** Over 50% of knee injuries involve anterior cruciate ligament (ACL) tears, which often lead to knee instability. Stem cell therapy has shown promise as an adjuvant treatment due to its self-renewal, differentiation, and immunomodulatory abilities. However, no comprehensive evaluation has been conducted to assess its effectiveness in promoting ACL healing. Therefore, this meta-analysis aimed to evaluate the potential of stem cell injections in enhancing ACL recovery after graft repair. ***Methods:*** This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Literature searches were performed up to July 2024 across databases such as ScienceDirect, PubMed, Cochrane, and Google Scholar using selected keywords related to stem cells and ACL repair. ***Results:*** From the search, six relevant studies were included. All studies used a controlled laboratory design; four employed in vivo models, and two combined in vivo and in vitro designs. Most utilized rabbit samples (four studies), while two used the flexor digitorum longus tendon. Bone marrow–derived mesenchymal stem cells (BMSCs) were the most common cell type, predominantly derived from isolated sources, with intervention sites mainly intra-articular or intratunnel. ***Conclusion:*** According to its ability to self-renew, differentiate, and have immunomodulatory effects, stem cell therapy has demonstrated encouraging outcomes when used as an adjuvant treatment. Because of their biomechanical characteristics, histological results, and magnetic resonance imaging, stem cells and their cytokines are well recognised to have numerous therapeutic benefits in promoting ACL healing following graft repair.

***Keywords:*** stem cell injections; Anterior Cruciate Ligament (ACL); graft repair.

## INTRODUCTION

Almost half of all knee injuries are caused by strains to the Anterior Cruciate Ligament (ACL), making it the most often injured ligament in the knee.[1–3] Over 50% of all knee injuries are ACL tears, which can lead to instability in the knee and eventually cause osteoarthritis and meniscal injury.[4] The most often torn ligament in the human body is ACL. Because of its close closeness to the ACL, it is frequently linked to tears of the medial and lateral collateral ligament fibers.[5]

A non-contact pivot injury, in which the tibia is moved anteriorly and the knee is slightly flexed and in a valgus position, is the most common mechanism by which ACL tears occur in sports. Another mechanism of injury that has been discovered is a direct hit to the lateral knee. Basketball, soccer, and skiers are the athletes most vulnerable to non-contact injuries. Football players are the athletes who are most susceptible to contact injuries.[1,6] ACL injuries have the potential to end an athlete's career and have a lasting impact on their level of physical activity. Injury to this ligament may impede

the joint's ability to move and be loaded normally. Anterior cruciate ligament injuries frequently need to be surgically repaired, then require extensive rehabilitation.[4]

A tendon graft is used in surgical regeneration of the ACL. The tendon's composition and structure are different from those of the ligament, with the ligament containing more proteoglycans and having a different distribution of collagen.[7] The self-renewal characteristics, differentiation potential, and immunomodulatory action of stem cell therapy have made it a promising adjuvant therapy.[8] It is commonly recognized that stem cells and the cytokines they release have a variety of therapeutic benefits that enhance the healing process of tendon injuries as well as the biomechanical characteristics of the tissue.[9] Three steps make up the tendon healing process: proliferative, remodeling, and inflammatory. These phases are comparable to those of graft ligamentation. Nevertheless, the application of stem cells to improve intra-articular graft healing has only been documented in a small number of investigations.[10] In addition to promoting healing by the secretion of different immunoregulatory molecules, such as paracrine trophic mediators, stem cells can develop into a variety of terminally differentiated lineages, which can be exploited to construct tissues produced from mesenchymal cells.[6]

Regarding the effectiveness of stem cell injections as Anterior Cruciate Ligament (ACL) therapy, several studies have shown inconsistent findings. But no organized and thorough evaluation has assessed the effectiveness of stem cell injections in improving Anterior Cruciate Ligament (ACL) healing following graft repair. Thus, the aim of this systematic review is to evaluate the potential of stem cell injections to improve Anterior Cruciate Ligament (ACL) healing following graft repair.

## METHODS

For this systematic review, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) are being used.[11] It was not necessary to obtain ethical approval for this study because it used already published research data.

### Study Selection

A literature search was conducted using the following keywords to uncover relevant topics up until July 2024 using databases including ScienceDirect, PubMed, Cochrane, and Google Scholar: "Anterior Cruciate Ligament" OR "ACL" AND "Stem Cell Injections" AND "Graft Repair".

### Inclusion and Exclusion Criteria

The following criteria were met in order for this meta-analysis study to be included: (1) observational or interventional studies, (2) studies regarding ACL reconstruction using a tendon graft with the injection of stem cells, (3) studies reporting biomechanical, histological, and/or MRI outcomes. The following were the study's exclusion criteria: (1) paper that does not use English; (2) conference papers, literature review, and case reports.

### Data Extraction

From the chosen studies, we took out the following information: (1) publication year and name of the first author; (2) country; (3) study design and study type; (4) samples, (5) graft type; (6) type, origin, and source stem cell; (7) intervention location. The outcomes collected include biomechanical evaluation, namely stiffness, ultimate failure load, and type III collagen content, histological evaluation, and MRI evaluation.

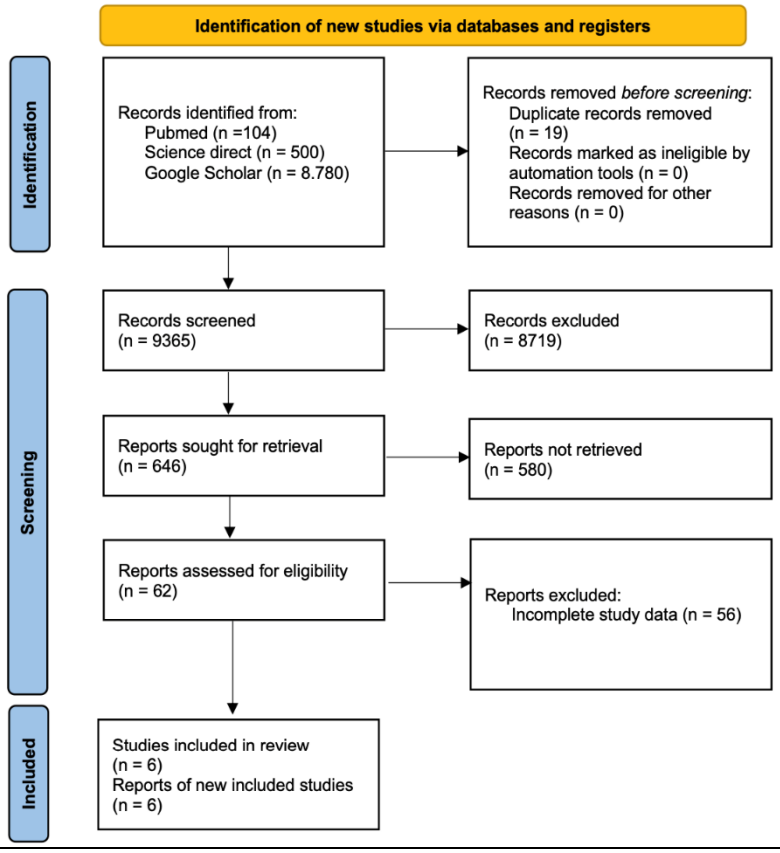


**FIGURE 1:** PRISMA Flowchart.

## RESULTS

### Study Characteristic

Based on the results of literature searches from 3 databases, 6 studies were obtained (Table 1). Based on their characteristics, 4 studies came from China, 1 study from Indonesia, and 1 study from Taiwan. Based on the study design, all six studies used a controlled laboratory study design. All studies were in vivo, while 2 studies were in vivo and in vitro. Most of the samples used rabbit samples (4 studies), while 2 other studies used rats. Based on graft type, 2 studies used the flexor digitorum longus tendon, 1 study used the femoral tendon, other studies used the hamstring tendon, bone–patellar tendon–bone, and the Achilles tendon. Based on the type of stem cell, most studies used BMSC (bone marrow–derived mesenchymal stem cell), while other studies used TDSC (tendon-derived stem cell). Based on the origin of the stem cell, most were rabbits, and the majority came from isolated cells. The majority of intervention locations were intra-articular and intratunnel.

### Biomechanical Evaluation

Biomechanical evaluation in this systematic review found results related to aspects of stiffness, ultimate failure load, and type III collagen content. According to the Chen et al, upon reconstruction, the AdEGFP group's stiffness values were much lower than those of the AdBMP2, AdbFGF, and AdBMP2-plus-AdbFGF groups. At 4 or 12 weeks, there was no significant difference between the AdBMP2 and AdbFGF groups. However, the AdBMP2-plus-AdbFGF group showed significantly more stiffness than the AdBMP2 and AdbFGF groups.[12] In the Lu study, both BMMNCs and BMSCs induced more inflammatory factors early on, as demonstrated by the higher MCP1 expression in BM cells caused two weeks after surgery. This could potentially reduce surrounding cell migration and proliferation in the graft with interfacial broader fibrocartilage formation following allograft ACLR.[13] Based on the Lui study, with reduced cellularity and vascularity, improved cell alignment, and increased collagen birefringence, semiquantitative image analysis demonstrated superior graft osteointegration and intra-articular graft integrity in the TDSC group. Significant differences were seen between the TDSC group's ultimate load at week 2 (52.5% increase, P =0.027) and stiffness at week 6 (62% increase, P =0.008).[14] According to Setiawati et al, the tendon graft tunnel's ultimate tensile strength and biomechanical analysis were connected. At 3 and 6 weeks, the mean ultimate tensile strength was considerably higher in the treated group than in the control group. The treated knee outperformed the control knee in terms of ultimate tensile strength on average by 36% and 15% at the 3- and 6-week time points, respectively.[15] Based on the Sun et al, the maximum failure load of the CM group was substantially larger than that of the NI group or the CI group at both 4 and 8 weeks following surgery. In terms of stiffness, the CM group's values at 4 and 8 weeks were both greater than those of the other 2 groups. However, statistical significance was only attained when comparing the CM group's values at 4 and 8 weeks to those of the NI group at 8 weeks and the CI group at 4 and 8 weeks.[16] Based on the Wei et al study, at 6, 12, and 24 weeks after surgery, the biomechanical parameters representing stiffness and ultimate failure load were found to be significantly higher in the TGFb1 group compared to the control group. A comparison of the VEGF165 or TGFb1/VEGF165 co-expression groups and the control group was made twelve weeks after surgery, and no significant changes were found. Nevertheless, the former had a higher ultimate failure load and stiffness. When it came to ultimate failure load and stiffness after 24 weeks, the TGFb1/VEGF165 group outperformed the other three.[17]

### Histological Evaluation

According to Chen et al, the majority of the AdEGFP group's histology revealed some irregularly ordered porous fibrous tissues between the bone tunnel and tendon. Along the bone tube, there was a small area of freshly created matrix in the AdBMP2 group that resembled a chondro-osteoid. The AdbFGF group showed freshly created vessels in the graft substance after the establishment of a large fibro-vascular interface and perpendicular collagen fibres along the load axis. A wide area of freshly created matrix resembling chondro-osteoid, together with a few big vessels, was observed at the contact in the AdBMP2-plus-AdbFGF group.[12] According to a histological analysis of the proximal tibia based on the Lu et al study, the tendon-bone interface was invaded by the intra-articularly injected cells. Wider gaps with interfacial fibrocartilage repair, equivalent collagen I levels, and increased MCP1 expression in the early stage were observed in the BM cell groups in comparison to the control group.[13] According to the Lui et al, in comparison to the control group, the intra-articular graft midsubstance in the TDSC group at weeks 6 and 12 displayed more collagen birefringence, improved cell alignment, and decreased cellularity and vascularity overall.[14] Based on H&E staining in the Sun et al study, cell infiltration and collagen fibre density increased in all three groups with respect to the graft midsubstance between 4 and 8 weeks after surgery. At eight weeks, the CM group had more organised collagen fibres. Using polarised microscopy, birefringence was found, which allowed for the quantification of the collagen fibril remodelling process. The sirius red staining revealed that the content of Col 1 increased between groups between 4 and 8 weeks postoperatively, suggesting a remodelling progress. However, the CM group showed a comparatively larger area of Col 1 birefringence than the NI group.[16] According to Wei et al, at 3,6,12, and 24 weeks following surgery, biomechanical characteristics, vascular number, and HE and toluidine blue staining were examined. The findings imply that TGFb1 expression in TGFb1 /VEGF165-transfected BMSCs may hasten the remodelling process of the ligament that has been rebuilt. TGFb1/VEGF165-transfected BMSCs significantly increased angiogenesis of the rebuilt ligament at 3, 6, and 12 weeks, with the best mechanical qualities being attained at 24 weeks,

demonstrating the favourable effects of the cross-talk between TGFb1 and VEGF165.[17]

**MRI Evaluation**

MRI evaluation was only reported by the study of Setiawati et al. Based on the study of Setiawati et al., graft maturation and MRI signal strength were associated. At three and six weeks, the treatment group's mean signal intensity score was considerably higher than that of the control group. When comparing the treated groups with the control groups at the 3- and 6-week follow-up, the graft signal intensity in the femoral tunnel gradually increased, which was consistent with a steady decrease in the interface and tunnel diameter.[15]

**TABLE 1:** Characteristic Study.

| Study | Year | Country | Study Design | Study Type | Samples | Graft Type | Stem Cell | | | Intervention Location |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Type | Origin | Source | |
| Chen et al | 2016 | China | Controlled laboratory study | In vivo, in vitro | Rabbit | Femoral tendon | BMSC | Rabbit | Purchased from the Laboratory Animal Center | Intra-articular and intratunnel |
| Lu et al | 2021 | Taiwan | Controlled laboratory study | In vivo | Rabbit | Hamstring tendon | BMSC | Rabbit | Isolated from iliac crest | Intra-articular |
| Lui et al | 2014 | China | Controlled laboratory study | In vivo | Rat | Flexor digitorum longus tendon | TDSC | Rat | Isolated from patellar tendon | Intra-articular and intratunnel |
| Setiawati et al | 2017 | Indonesia | Controlled laboratory study | In vivo | Rabbit | Bone– patellar tendon–bone | BMSC | Rabbit | Aspirated from the pelvic bone | Intra-articular and intratunnel |
| Sun et al | 2019 | China | Controlled laboratory study | In vivo, in vitro | Rat | Flexor digitorum longus tendon | BMSC | Human | Purchased from ScienCell Research Laboratories | Intra-articular |
| Wei et al | 2011 | China | Controlled laboratory study | In vivo | Rabbit | Achilles tendon | BMSC | Rabbit | Isolated from bone marrow by drilling a hole into femur and tibia | Intra-articular and intratunnel |

**TABLE 2:** Outcome of The Study.

| Study | Year | Week | Biomechanical Evaluation | | | | | | Histological Evaluation | | MRI Evaluation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stiffness | | Ultimate Failure Load | | Type III collagen content | | | | | |
| | | | Results | P | Results | P | Results | P | Results | P | Results | P |
| Chen et al[12] | 2016 | 4 | N/A | <0.01 | N/A | N/A | N/A | N/A | • The majority of the AdEGFP group's histology revealed some irregularly ordered porous fibrous tissues between the bone tunnel and tendon.<br>• Along the bone tube, there was a small area of freshly created matrix in the AdBMP2 group that resembled a chondro-osteoid. The AdbFGF group showed freshly created vessels in the graft substance after the establishment of a large fibro-vascular interface and perpendicular collagen fibres along the load axis.<br>• A wide area of freshly created matrix resembling chondro-osteoid, together with a few big vessels, were observed at the contact in the AdBMP2-plus-AdbFGF group. | N/A | N/A | N/A |
| | | 12 | N/A | <0.01 | N/A | N/A | N/A | N/A | • The AdEGFP showed a progressive restoration of collagen fibre continuity between the tendon and bone, perpendicular to the load axis.<br>• In the AdBMP2, AdbFGF, and AdBMP2-plus-AdbFGF groups, a shift from tendon to non-mineralized fibrocartilage and mineralised cartilage was seen. The AdBMP2-plus-AdbFGF group also exhibited a larger region of cartilage-like cells than either the AdBMP2 or AdbFGF group. | N/A | N/A | N/A |
| Lu et al[13] | 2021 | 2 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0.013 | N/A | N/A |
| | | 6 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0.027 | N/A | N/A |
| Lui et al[14] | 2014 | 2 | N/A | N/A | TDSC: 11.2<br>Control: 7.4 | 0.027 | N/A | N/A | TDSC: graft intact<br>Control: cellularity and vascularity increased | N/A | N/A | N/A |
| | | 6 | TDSC: 37.0<br>Control: 23.0 | 0.08 | N/A | >0.05 | N/A | N/A | TDSC: lower cellularity and vascularity<br>Control: NA | N/A | N/A | N/A |

| Study | Year | Week | Biomechanical Evaluation | | | | | | Histological Evaluation | | MRI Evaluation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stiffness | | Ultimate Failure Load | | Type III collagen content | | | | | |
| | | | Results | P | Results | P | Results | P | Results | P | Results | P |
| | | 12 | TDSC: 48.7<br>Control: 39.3 | 0.441 | TDSC: 26.4<br>Control: 15.7 | 0.149 | N/A | N/A | TDSC: lower cellularity and vascularity<br>Control: NA | N/A | N/A | N/A |
| Setiawati et al[15] | 2017 | 3 | N/A | N/A | BMSC: 0.99 ± 0.12<br>Control: 1.35 ± 0.11 | 0.001 | BMSC: 8.63 ± 2.48<br>Control: 4.00 ± 2.13 | | N/A<br>N/A | N/A<br>N/A | Signal Intensity Score: BMSC: 7.17 ± 0.75; Control: 3.33 ± 0.52<br>Tunnel Diameter: BMSC: 1.97 ± 0.05 Control: 2.07 ± 0.04<br>Interface Diameter BMSC: 0.50 ± 0.03 Control: 0.61 ± 0.05 | N/A |
| | | 6 | N/A | N/A | BMSC: 1.28 ± 0.04<br>Control: 1.47 ± 0.12 | 0.014 | BMSC: 10.10 ± 2.13<br>Control: 5.32 ± 1.75 | | N/A | N/A | Signal Intensity Score BMSC: 8.83 ± 0.40 Control: 3.5 ± 1.22<br>Tunnel Diameter BMSC: 1.98 ± 0.03 Control: 2.14 ± 0.05<br>Interface Diameter BMSC: 0.48 ± 0.03 Control: 0.59 ± 0.02 | N/A |
| Sun et al[16] | 2019 | 4 | CM: 9.00 ± 1.57<br>Control: 7.09 ± 1.32 | 0.029 | CM: 5.68 ± 1.13<br>Control: 3.81 ± 0.76 | 0.001 | N/A | N/A | Type I collagen content CM: 256.25% ± 53.17%<br>Control: 103.63% ± 18.24% | <.001 | N/A<br>N/A | N/A<br>N/A |
| | | 8 | CM: 12.83 ± 2.04 | <0.001 | CM: 14.91 ± 1.54 | <0.001 | N/A | N/A | Type I collagen content CM: 598.75% ± 142.67% | | N/A | N/A |

| Study | Year | Week | Biomechanical Evaluation | | | | | | Histological Evaluation | | MRI Evaluation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stiffness | | Ultimate Failure Load | | Type III collagen content | | | | | |
| | | | Results | P | Results | P | Results | P | Results | P | Results | P |
| | | | Control: 8.51 ± 1.70 | | Control: 10.21 ± 1.12 | | | | Control: 250.40% ± 61.38% | <.001 | N/A | N/A |
| Wei et al[17] | 2011 | | | | | | | | No. of microvessels | | | |
| | | 3 | N/A | >0.05 | N/A | >0.05 | N/A | N/A | VEGF-165: NA<br>Coexpression: NA<br>Control: NA | <0.05 | N/A | N/A |
| | | 6 | N/A | <0.05 | N/A | <0.05 | N/A | N/A | VEGF-165: NA<br>Coexpression: NA<br>Control: NA | <0.05 | N/A | N/A |
| | | 12 | N/A | <0.05 | N/A | <0.05 | N/A | N/A | VEGF-165: NA<br>Coexpression: NA<br>Control: NA | <0.05 | N/A | N/A |
| | | 24 | N/A | <0.05 | N/A | <0.05 | N/A | N/A | VEGF-165: NA<br>Coexpression: NA<br>Control: NA | >0.05 | N/A | N/A |

**DISCUSSION**

When determining the best time for ACL reconstruction, three key considerations should be weighed. The danger of arthrofibrosis connected with an early ACL reconstruction, the higher incidence of meniscus and chondral injuries after a delayed procedure, and the resulting loss of muscle strength from inactivity can all be factors.[18] The patellar tendon (PT), sometimes referred to as a BPTB graft, and the hamstring tendon (HT) are the two autografts that are most frequently used for ACL restoration. Biomechanical data comparing the PT graft and HT with native ACL revealed that the PT graft had a stiffness of about 57 MPa, which translates to being between roughly 160 and 170% stronger and 150% stiffer than native ACL. Its maximum load was 2730 N or 2900 N, depending on whether the central or middle portion of BPTB was tested.[18] The PT graft's claimed decreased re-tear rate when compared to hamstring grafts is one of its primary benefits. A meta-analysis indicates that the incidence of graft failure for PT grafts is 1.9%, while for HT grafts it is 4.9%. This suggests that PT grafts have a lower failure rate.[18]

For ACL restoration, the best graft is one that is easily harvested, has minimal morbidity at the harvest site, integrates well with the bone, and is biomechanically similar to the native ligament. Every graft has benefits and drawbacks, hence there is currently no perfect graft for ACL restoration. Compared to other forms of grafts, autografts are utilised more frequently. Bone patellar tendon bone (BPTB), hamstring, and bone quadriceps tendon grafts are the three autograft types that are most frequently utilised. In the past, BPTB was regarded as the best method for reconstructing the ACL. Because of the BPTB autograft's superior clinical results and high patient satisfaction rate, it is widely selected. One of the most popular grafts for ACL restoration is the hamstring tendon transplant.[19–22] With or without the gracilis tendon, the ipsilateral leg is used to harvest the semitendinosus tendon. The donor site morbidity associated with hamstring grafts is low, but the healing and extension of the bone-tendon junction is problematic. Although allografts don't work well for immunity or re-rupture rates, they can be employed for revision or multiligamentous injuries. There is currently no ideal synthetic graft available; synthetic grafts are continually evolving.[7]

The extraordinary capacity of stem cells for multilinear culture, long-term survival, and self-renewal makes them indispensable for tissue engineering technologies. In tissue engineering, a variety of cell sources are frequently used, including induced pluripotent stem cells (iPSCs), adipose tissue-derived stem cells (ADSCs), bone marrow-derived mesenchymal stem cells (BMSCs), and tendon/ligament stem/progenitor cells (TDSCs /LDSCs). MSC in particular is attracting a lot of attention because it is simple to identify and cultivate these cells in vitro from a range of adult tissues.[6]

Without the use of growth hormones, mechanical loading has been shown to affect cell division, apoptosis, proliferation, and the creation of extracellular matrix. It has been demonstrated that mechanical stimulation increases ECM deposition and cell proliferation in fibroblasts. It increases fibrocartilage and mechanics to promote tendon-bone repair following ACLR. Collagen I/III, alkaline phosphatase, osteopontin, and tenascin C were expressed at higher levels in vitro in BMSC/TC cocultures triggered by mechanical stretch than in BMSC alone. Indeed, the mechanical stimulation's duration, direction, intensity, and frequency would all affect the state of the cells. The expression of fibronectin, collagen type II, and collagen type I was reduced by early mechanical stress on MSCs, but it was increased throughout the proliferation stage.[22–26]

BMSCs have been extensively researched for improving tendon-bone repair, with excellent results, due to their multipotential ability to differentiate into osteoblasts, chondrocytes, and adipocytes. Comparing the proliferative capacities of several stem cell types, Sakaguchi found that whereas ADSCs lost their proliferative capacity at passage 7, BMSCs remained stable until passage 10. Since there is a chance of donor damage and ectopic ossification, these cells are not thought to be the best option. Based on their migration to inflammatory areas and subsequent decrease of inflammation, BMSCs are hypothesised to have therapeutic effects. In the course of tissue repair mechanisms, they are rarely involved in colonising the healing tissue.[23,24] The previous study revealed that tendon fragments as small as 1 μm could potentially contain TDSCs. Previously, TDSCs had only been isolated from the human hamstring tendon. These cells outperformed BMSCs in terms of multilineage differentiation capability, clonogenicity, and immunogenicity.[27–31]

The included studies used stem cell delivery techniques such as intra-articular direct injections, stem cell sheet wrapping, and intra-articular CM injections to encourage graft ligamentization. Six investigations described direct stem cell injections intra-articularly. Although direct intra-articular stem cell injection is frequently done, there are still questions about its safety, particularly with regard to tumorigenicity. In one study, a TDSC sheet was employed. The usage of synthetic scaffolds, which may have issues with immunogenicity, biocompatibility, and biodegradability, was avoided by the stem cell sheet. Surgeons are still having difficulty implanting and fixing a stem cell sheet on the transplant, though. In addition to exosomes, tem cell CM carries a range of growth factors, such as VEGF, TGF-β, and insulin-like growth factor.[10,32,33]

The function of BM-derived cells was dual in nature, as they supported the development of neutrophils, eosinophils, basophils, mast cells, and monocytes into inflammatory cells while also inducing tissue repair (non-hemopoietic cells).

Moderate inflammation is essential for tendon/ligament healing during tendon repair and reconstruction, particularly for macrophage polarisation to support tissue remodelling, scar resolution, and immunosuppression. Prolonged inflammation, on the other hand, causes excessive remodelling and scar repair.[34] In one study, Lu et al. directly injected autologous BM and BMSCs into the extra-articular tendon-bone tunnel in a rat model. They observed that the autologous BM group had more interfacial organisation of fibres with increasing stiffness and failure load than the BMSCs group due to increased M2 macrophage polarisation.[13]

Following an ACL injury, growth factors and cytokines are crucial for tissue repair. In animal models of ACLR, platelet-rich plasma (PRP) has been demonstrated to improve the quality of graft tissue, leading to improved biomechanical qualities and greater expression of collagen. Additionally, PRP stimulates tissue regeneration because it inhibits inflammation via a variety of signalling pathways, releases a variety of anti-inflammatory chemicals, and modifies macrophage subtypes. Extracellular vesicles (EVs) are novel biomaterials used in tissue regeneration that are derived from stem cells and include paracrine factors. In a model of patellar tendon injury, Shi et al. showed that EVs promote tendon repair, attenuate inflammation by upregulating the production of anti-inflammatory mediators, and direct BMSCs towards tenogenic differentiation.[35,36] Additionally, in the early stages of mice Achilles tendon-bone restoration, EV administration may improve tendon-bone healing by polarising more M2 macrophages and reducing the population of M1 macrophages as well as proinflammatory factors. In the early stages following BM cell injection, our study's raised expression of MCP1 suggested increased inflammation at the tendon-bone interface.[35,36]

The current review has three limitations even though it was carried out in compliance with PRISMA principles. The majority of the listed investigations were conducted in vivo. The included research used ACL reconstruction models from rats and rabbits. Regarding the mechanical characteristics and stability of ACL transplants, these tiny animals differ from humans. The function of stem cells in fostering graft ligamentization following ACL surgery requires more study.

## CONCLUSION

According to its ability to self-renew, differentiate, and have immunomodulatory effects, stem cell therapy has demonstrated encouraging outcomes when used as an adjuvant treatment. Because of their biomechanical characteristics, histological results, and magnetic resonance imaging, stem cells and their cytokines are well recognised to have numerous therapeutic benefits in promoting ACL healing following graft repair. Therefore, in order to assess the impact of stem cell injection in ACL healing following graft replacement, more research with varied designs and larger human samples is required.

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