

REVIEW ARTICLE

In Vivo Studies of Mesenchymal Stem Cells in the Treatment of Meniscus Injury

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This review summarizes the literature of preclinical studies and clinical trials on the use of mesenchymal stem cells (MSCs) to treat meniscus injury and promote its repair and regeneration and provide guidance for future clinical research. Due to the special anatomical features of the meniscus, conservative or surgical treatment can hardly achieve complete physiological and histological repair. As a new method, stem cells promote meniscus regeneration in preclinical research and human preliminary research. We expect that, in the near future, *in vivo* injection of stem cells to promote meniscus repair can be used as a new treatment model in clinical treatment. The treatment of animal meniscus injury, and the clinical trial of human meniscus injury has begun preliminary exploration. As for the animal experiments, most models of meniscus injury are too simple, which can hardly simulate the complexity of actual meniscal tears, and since the follow-up often lasts for only 4–12 weeks, long-term results could not be observed. Lastly, animal models failed to simulate the actual stress environment faced by the meniscus, so it needs to be further studied if regenerated meniscus has similar anti-stress or anti-twist features. Despite these limitations, repair of the meniscus by MSCs has great potential in clinics. MSCs can differentiate into fibrous chondrocytes, which can possibly repair the meniscus and provide a new strategy for repairing meniscus injury.

Key words: Cytology treatment; Meniscus injury; MSCs

Introduction

The meniscus of the knee is a wedge-shaped fibrocartilaginous tissue with a thick peripheral rim and a thin inner rim. The menisci are attached between the femoral condyle and tibial surface, with the medial meniscus covering 60% of the tibiofemoral surface and lateral meniscus covering 90% of the articular surface¹. Water and collagen tissues constitute 72% and 22%, respectively, of the composition of the meniscus. Out of the 22% collagen tissue, 80% is type I collagen and 20% is type II collagen². Notably, the distribution of collagen types differs, as type I collagen fibers form a network structure filled with interstitial cells, glycosaminoglycans, glycoproteins, and elastin³. The phenotype of interstitial cells has more fibroblast-like cells in the peripheral and more chondrocyte-like cells in the inner rim⁴. The main functions of the meniscus are to spread load,

reduce contact stress, absorb impact, lubricate joints, and maintain proprioception⁵.

Annular capillaries formed by upper and lower arteries can provide limited blood flow for the tissue in 10%–25% of the peripheral meniscus rim, 10%–30% of the inner meniscus rim, and in the anterior and posterior horns of the meniscus⁶. When these capillaries enter the meniscus, they are normally arranged into three layers—upper, middle, and lower layers—and this forms the basis for self-repair after meniscus injury. Tears are located at the peripheral attachment sites. However, in the remaining parts of the meniscus, there is no clear vascular nourishment, yet a membranous synovial layer covers the surface of the meniscus and provides synovial fluid, which is a major source for the limited growth repair of the tissue⁶. Anderson *et al.*⁷ recommended a classification system of zone 1 (rim width <3 mm), zone

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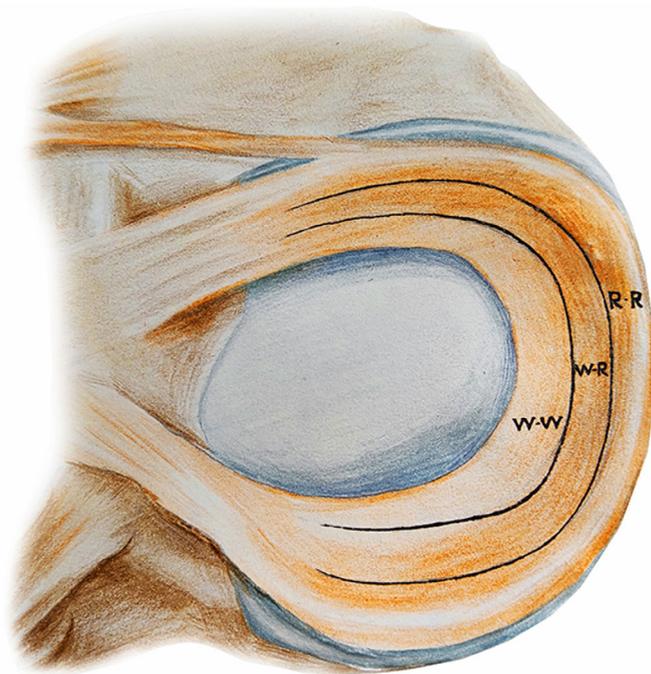


Fig 1 Illustration of meniscus demonstrating the classic three zones according to the reported vascularity. R-R, red-red; R-W, red-white; W-W, white-white. The R-R zone has a good blood supply, if the tear is very small, sometimes it can heal on its own and may not necessarily require surgical treatment. The R-W zone has partial blood supply and its self-healing potential is low. There is almost no blood supply in the W-W zone, and a tear in this area will not heal by itself.

2 (rim width 3 to <5 mm), and zone 3 (rim width \geq 5 mm). Meniscofemoral and meniscotibial, or zone 1, are also commonly referred to as outer third, or red-red (R/R), tears. Tears located in the middle third (zone 2) are classified as red-white (R/W) tears, and tears in the inner third (zone 3) are termed white-white (W/W) tears. R/W tears occur at the junction of the outer and middle third regions, approximately 4 mm from the meniscal attachment, with a vascular supply predominately in the outer third of the tear. W/W tears are located in the region in which no blood supply exists⁸ (Fig. 1).

Meniscus injuries are associated with the shape of injury and state of stress during injury. The types of meniscus injury include oblique (parrot-beak), horizontal (spallation), radial, vertical (longitudinal), and bucket handle-like tears (Fig. 2). An oblique (parrot-beak) tear is a kind of minor meniscus injury, which tears from the free edge of the meniscus to the middle of the meniscus, affecting the superior and inferior surfaces. If the injury tears throughout the whole meniscus, it should be called a radial tear. The meniscus with a horizontal (spallation) tear is just like puff pastry, tears across the central layer, but may not affect the surface, while a vertical (longitudinal) tear is parallel to the edge of the meniscus. The type of injury can exist either in the red or white zone, and if accompanied by a flip of the free edge

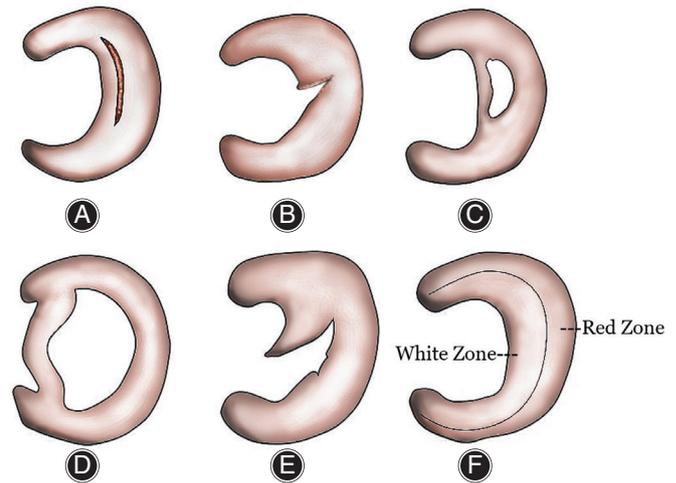


Fig 2 Anatomical structure of meniscus and type of injury. The types of meniscus injury include vertical (A), radial (B), horizontal (C), bucket handle-like tears (D), and oblique (E). Annular capillaries formed by the upper and lower arteries can provide limited blood flow for the tissue in 10%–25% of the lateral meniscus rim, 10%–30% of the medial meniscus rim, and in the anterior and posterior horns of the meniscus (Red Zone, F). The tears in the white zone have limited ability to self-repair; patients may suffer persistent and repeated symptoms, as well as aggravated injuries, due to the lack of self-healing.

of the meniscus, it should be called bucket handle-like tear. As mentioned earlier, there is a relationship between the ability of meniscus repair and the type of injury. Some injuries, such as oblique (parrot-beak) tears and radial tears, normally cross the white zone, and they do not cure easily, even when stitching the meniscus with sutures or Fast-fix. The bucket handle-like tear is a severe type of meniscus injury.

As are characteristics of our description of the meniscus blood supply, these tears have limited ability to self-repair. Patients may suffer persistent and repeated symptoms, as well as aggravated injuries due to the lack of self-healing. Besides, there is a high failure rate of 18% (16% for single longitudinal type and 28% for double longitudinal type) for surgery to repair meniscal avascular zones⁸, and thus partial resection of the meniscus has become the treatment of choice in clinics. Studies have shown that even in cases where meniscus injury is combined with anterior cruciate ligament injury, partial resection of the meniscus can lead to a 10–20-fold increase in the incidence of lateral knee osteoarthritis^{9–11}. The incidence of osteoarthritis is even higher among patients with complete meniscus resection. Long-term follow-up showed that the knee force line of patients had changed, and their joint space had varying degrees of stenosis. This was particularly prominent for patients with a body mass index (BMI) >30 ^{12–14}. It has been suggested that the femorotibial joint is inherently a completely mismatched surface where the stress of the joint is focused on a very small area, and this is extremely unfavorable for the transfer

of load¹⁵. However, the presence of menisci makes the joint a mildly mismatched surface through self-filling, and partial or complete resection of the meniscus would inevitably lead to reduced stress-sharing, resulting in further degenerative changes of articular cartilage due to the focused stress.

Therefore, our systematic review compiled multiple studies with the aim to: (i) review *in vivo* experiments for the treatment of meniscus injury (particularly in the avascular zones) using MSCs; (ii) evaluate the efficacy of MSCs from different sources in the treatment of meniscus injury in animals and humans; and (iii) find the evidence to support that the intra-articular injection of stem cells can be used as a new method for the treatment of meniscus injury.

Data and Methods

A literature search was performed in PubMed including the following keywords: meniscal repair, meniscal injury, biomaterials, and stem cells. A literature search from 1997 to 2017 was conducted and all studies evaluating development and application were included in the review. The inclusion criteria were: (i) article published recently in an authoritative magazine, including periodical paper, academic paper, and review; (ii) content of article is closely related to meniscus injury and stem cells and is highly recognized by joint surgeons; (iii) study design is a prospective or retrospective comparative study; (iv) experiment objects, injections, histological findings, and clinical outcomes were compared; and (v) humans or animals with meniscus injury. The exclusion criteria were as follows: (i) unable to obtain full text; (ii) repeat studies; and (iii) low evidence level. If the article was in line with the topic mentioned above, the full text was accessed, and the article was read in detail and included in this review.

There were 694 records identified through database searching; 398 records were excluded and only 34 articles were included in the qualitative synthesis. In all, 32 articles were on animal research and the others were on human research (Fig. 3).

Current Status of Cytological Treatment of Meniscus Injury

Cytological treatment of the meniscus induces meniscus fibroblasts to differentiate and mature. Recent technologies have induced differentiation of MSCs (Fig. 4). MSCs are pluripotent stem cells that are distributed in many human organs, including skeletal muscles, pancreas, adipose tissues, placenta, bones, and bone marrow. Regardless of the source, all MSCs have similar abilities in osteogenesis, adipogenesis, and chondrogenesis^{16,17}. MSCs normally used in clinics are derived from bone marrow, adipose tissue, umbilical cord blood, and dental pulp.

Researchers¹⁸ have compared the ability of different MSCs (derived from the bone marrow, meniscus, synovium, adipose tissue, muscles, ligaments, and periosteum) used most commonly for the meniscus and found that marrow-derived MSCs had the highest differentiation potential

followed by synovium-derived MSCs. However, ease of retrieval, size of the trauma, and the range of selectable materials should also be considered when deciding on the source of cells in the clinics. Due to these factors, adipose MSCs have irreplaceable advantages¹⁹.

Animal Experiments

More and more researchers began to focus on the ability of MSCs to repair meniscus injury *in vitro* (Table 1).

Synovial MSCs in Meniscus Repair

A study conducted by Mizuno *et al.*²⁰ injected synovial MSCs into the joint cavity of mice with meniscus and cartilage injuries and found the cylindrical defect of a meniscus filled with fibrous cartilage-like tissues. The control group was untreated, and its size of meniscal defects was significantly larger than that of the experimental group. Similar filling of the defect was also seen in the cartilage modeling group. This indicates that the injection of uninduced synovial MSCs into the joint cavity could promote meniscus and cartilage repair in mice, and the migration of stem cells is possibly driven by inflammatory reactions. Such pro-inflammatory migration has been confirmed in many animal experiments^{21–25}.

Immunofluorescence-labeled synovial MSCs were injected into mice and rat models with meniscus resection, and the area of meniscus defects in the experimental group was found to be significantly smaller than that in the control group. Labeled stem cells were identified in regenerated meniscal tissues with a morphology similar to that of fibrochondrocytes^{26,27}. However, no similar stem cells were found in other tissues or organs.

A study conducted by Zellner *et al.* divided New Zealand white rabbits into four groups and resected the anterior horn and body of the meniscus²⁸. Experimental groups 1, 2, and 3 were given injections of synovial MSCs, autologous platelet-rich plasma, and autologous bone marrow into the joint cavity. After 12 weeks of growth, the four groups showed varying degrees of healing, and the one treated with MSCs had the largest area of meniscus when compared with the others. This indicated that MSCs could promote the healing of meniscus avascular zones in rabbits. Rabbits treated with bone marrow or platelet-rich plasma showed no significant difference when compared with the control.

Rhesus macaques at 12–13 years old were used to simulate meniscus injury in aged primates²⁹. First, they cut the transverse ligament at the anterior horn of the meniscus, freeing the anterior horn and the body of the meniscus. Then, they removed half of the meniscus by cutting it at the level of the medial collateral ligament. Subsequently, the autologous synovial MSCs were cultured into aggregates and transplanted onto the meniscus defect of one knee (average number of cells: 3×10^6), while the other knee was left untreated. After the surgery, the rhesus macaques were allowed free activity to simulate human activity as much as

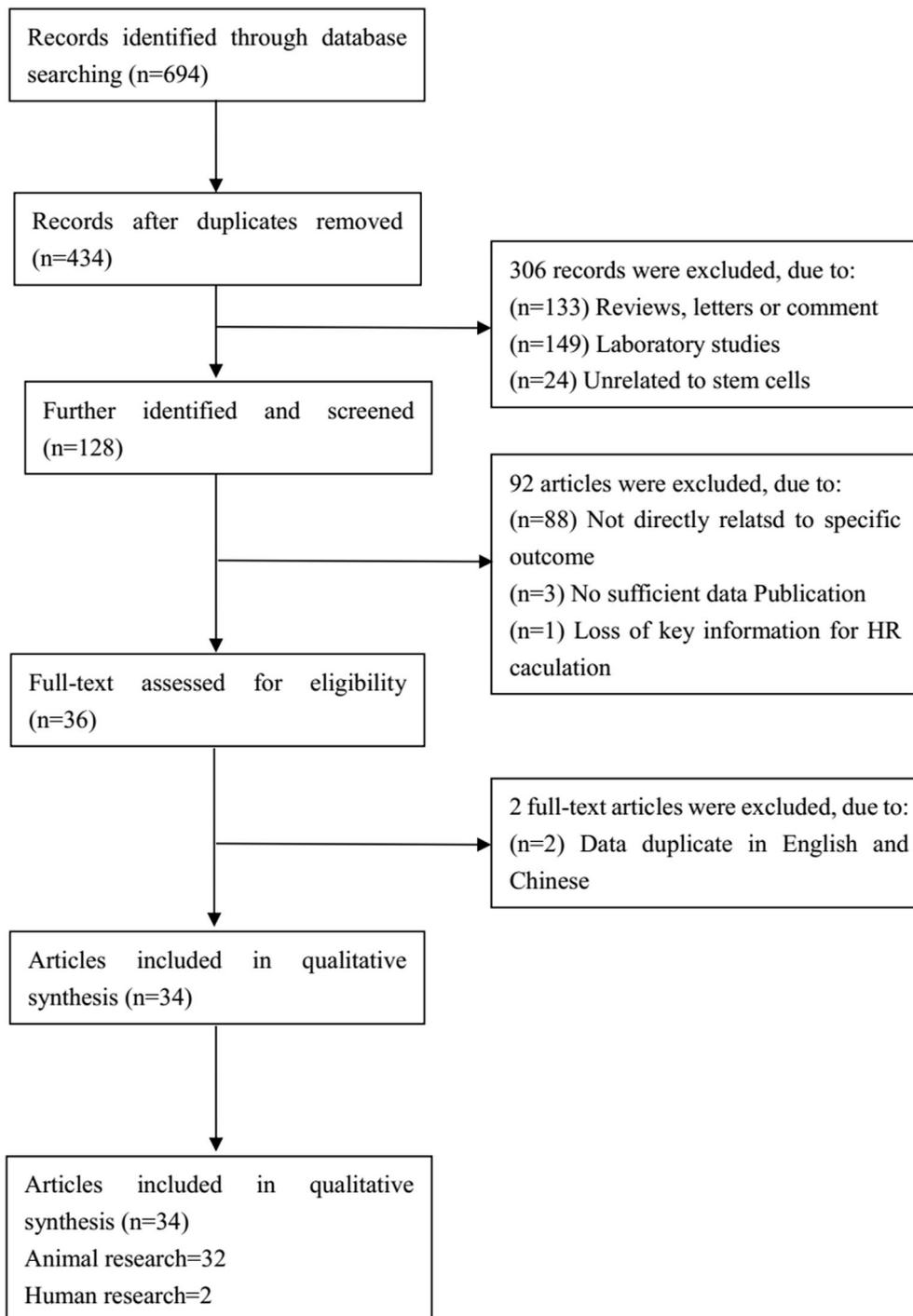


Fig 3 Retrieval process for literature for this article includes five inclusion criteria and three exclusion criteria. A literature search from 1997 to 2017 was conducted and all studies evaluating development and application were included in the review. There were 694 records identified through database searching; 398 records were excluded and only 34 articles were included in the qualitative synthesis. In all, 32 articles were animal research, and the others were human research.

possible. Morphological observation, magnetic resonance imaging (MRI), and histological scoring were performed after 8 and 16 weeks post-surgery. Both knees showed healing of the defects, but the size of meniscus in the MSC group was significantly larger than that in the control group. Safranin staining was positive in the experimental group and negative in the control group, indicating rich mucopolysaccharide structures in the regenerated meniscus. This was

consistent with the morphology of the fibrous cartilage meniscus. MRI T1rho mapping showed that the T1rho values of the menisci in the experimental group were closer to the normal meniscus, suggesting that regenerated menisci in the experimental group have more similar components with the normal meniscus than those in the control group. For both the experimental and control groups, degeneration of articular cartilage was found in the load-bearing parts of

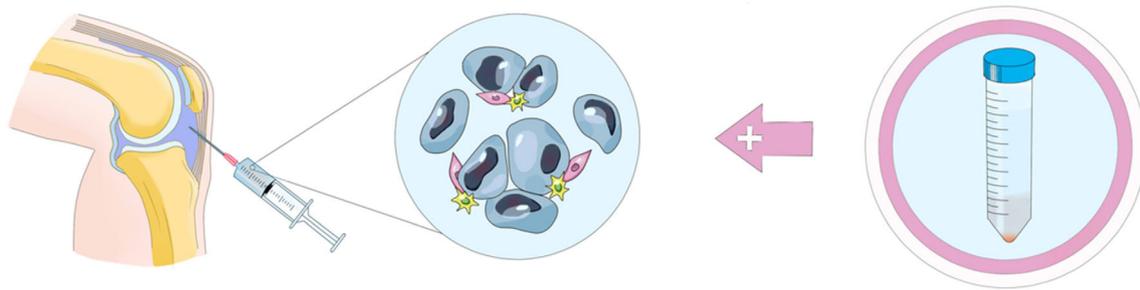


Fig 4 Cytological treatment of meniscus injury using MSCs. As the structural characteristics of the joint capsule, the knee joint is a relatively closed space. The prepared stem cell preparations can be injected directly into the joint cavity through the skin.

the femoral condyle. This degeneration was not significantly different between two groups 8 weeks after transplantation, but the area of cartilage degeneration in the experimental group was significantly smaller than the control after 16 weeks. This further revealed that meniscus resection could aggravate cartilage degeneration at related sites, and MSC repair of the meniscus could delay such degeneration. Also, stem cells may play a direct role in cartilage repair.

In addition to revealing the possibility of using synovial MSCs to repair menisci, Kondo *et al.*²⁹ also proposed the advantages of tissue engineering technologies to traditional cytology treatment. In Kondo's study, articular cartilage was degenerated in the MSC and control groups in all four primates at 16 weeks, while Mankin's score for histology was better in the MSC group compared to that in the control group in all four primates at 16 weeks. Katagiri³⁰ used synovial MSCs to treat meniscal defects in rats and stated that stem cell-material complexes are more advantageous than joint cavity injection due to the following reasons: (i) they possess higher potential in cartilage formation; (ii) they do not require an adhesive, as they can adhere to the meniscal surface by tension and gravity; and (iii) they can delay stem cell degeneration. Koch *et al.*³¹ showed that an accelerated repair of a combined avascular and vascular meniscus defect can be achieved by using a mesenchymal stromal cell-loaded porous polyurethane scaffold in a New Zealand white rabbit model, in which white rabbits were anesthetized using a combined intramuscular application of 0.6 mL/kg of ketamine 10% and xylazine 2% beforehand. Arthroscopic meniscectomy is still the main treatment at present, but it also increases the risk of osteoarthritis³². So, tissue engineering offers new treatment modalities for meniscus repair or even for meniscus replacement³³.

Bone Marrow MSCs in Meniscus Repair

Bone marrow MSCs were also used in early studies of mice, rabbit, goat, and other animals^{34–36}. These studies further confirmed that MSCs help to repair meniscus injuries. This repair can also restore the histological, mechanical, and morphological characteristics of defective menisci. Synovial and bone marrow MSCs can be obtained easily in animal

experiments, but the sampling of these cells may be difficult in clinics, leading to significant trauma. Thus, more and more studies are focusing on adipose MSCs that are of similar activity but can be isolated and sampled more easily with a relatively high survival rate. Ruiz *et al.* performed suture repair of the meniscus avascular zone tear in rabbits³⁷. After surgery, one side of the joint cavity was injected with allogeneic adipose MSCs and the possibility of meniscal repair increased 32-fold compared with the other cavity without injection. Qi *et al.* performed meniscus anterior horn resections in 18 New Zealand rabbits and randomly injected adipose MSCs (superparamagnetic iron oxide-labeled and non-labeled) and saline into their joint cavities³⁸. After the surgery, a magnetic field with the same intensity was placed around the rabbit knee to induce the labeled MSCs to migrate toward the area of meniscal defect. At the end of the study, pathological results showed that regenerated meniscus tissues of rabbits injected with adipose MSCs were similar to the normal rabbits, regardless of the labelling. The newly formed tissues showed the morphology of fibrous chondrocytes and were wrapped in massive extracellular matrix. There was no significant difference between the labeled and non-labeled groups. Prussian staining of the iron oxide revealed that the labeled stem cells were tightly adhered to the regenerated meniscus tissues. Apart from these findings, varying levels of cartilage degeneration were observed in all three groups. But, in the MSC group, the level of degeneration was significantly lower than that in the control group. Although labeled-migration induction seemed superfluous in this study, it did show that the low signals developed by magnetic materials can be detected by MRI, which provides new possibilities of chemotactic migration of MSCs.

With the development of tissue engineering, the choice of stem cell carriers has become a new concern. Pabbruwe *et al.* implanted human bone marrow MSC-absorbable collagen complex into the menisci of sheep and found that most uninduced MSCs showed good integration with meniscal fibrous chondrocytes. Biomechanical testing demonstrated a significant 2-fold increase in tensile strength in all constructs using the stem cell/collagen-scaffold compared to the control groups after 40 days in culture³⁹. However, MSCs induced

TABLE 1 Animal experiments

Time	Animal	Injection	Observation time	Histological findings
Mizuno <i>et al.</i> ²⁰ , 2007	Rats	1×10^7 synovial MSCs	12 weeks	Chondrocytes matured morphologically and gaps around the outside of the defect improved.
Zellner <i>et al.</i> ²¹ , 2013	New Zealand white rabbits	1×10^5 autologous MSCs-hyaluronic acid-collagen complex implantation	12 weeks	The repair tissue was meniscus-like with a low cell number, which showed typical meniscal pericellular cavities, and extensive amounts of extracellular matrix.
Al Faqeh H <i>et al.</i> ²¹ , 2011	Sheep	2×10^6 bone marrow MSCs	6 weeks	The H&E staining showed presence of cells and fibrous tissue in the meniscus-like tissue of the CM group and this feature resembles fibrocartilage as in native meniscus even though the cell looks immature with large nucleus.
Horie <i>et al.</i> ^{26,27} , 2011	Rats	5×10^6 synovium-MSCs	12 weeks	The contour of the regenerated menisci sharpened, and the ultimate forms were closer to the normal meniscal shape.
Zellner <i>et al.</i> ²⁸ , 2009	New Zealand white rabbits	1.5×10^6 synovial MSCs	12 weeks	The reconstitution of meniscus architecture with typically radial-orientated collagen fibers could be observed.
Kondo <i>et al.</i> ²⁹ , 2015	Primates	2.5×10^5 synovial MSCs	16 weeks	The regenerated meniscus was positively stained with safranin-O in the MSC group.
Katagiri ³⁰ , 2012	Rats	2.5×10^4 synovial MSCs	12 weeks	The contours of the regenerated menisci were sharper, and the stainability of type II collagen in the matrices was high.
Ruiz <i>et al.</i> ³⁷ , 2010	Rabbits	1×10^5 adipose MSCs	12 weeks	The healed areas presented a slight cellularity increase compared with the normal tissue.
Qi <i>et al.</i> ³⁸ , 2015	New Zealand white rabbits	2×10^6 adipose MSCs	12 weeks	The anterior portion of the meniscus was regenerated, with a greater mass of hypercellular fibrocartilaginous tissue and extracellular matrix (ECM).
Pabbruwe <i>et al.</i> ³⁹ , 2009	Sheep	3.5×10^5 human bone marrow MSC- absorbable collagen complex implantation	40 days	Extensive integration at one of the meniscal surfaces when using undifferentiated stem cells but revealed only limited integration when using differentiated cells.
Tatsuhira <i>et al.</i> ⁴⁰ , 2015	Japanese rabbits	5×10^4 adipose MSCs	12 weeks	Safranin-O staining was noted at 2 weeks and increased gradually over time until 12 weeks
Dutton <i>et al.</i> ⁴⁴ , 2009	Pigs	1×10^6 bone marrow MSCs	12 weeks	MSC-treated menisci with complete cellularity, fibrochondrocytes being the predominant cells.
Shen <i>et al.</i> ⁴⁶ , 2013	Rats	6×10^6 human meniscus progenitor cells	12 weeks	Intra-articular injection of hMeSPCs induced significantly more neo-tissue formation and extracellular matrix (ECM) deposition.
Okuro <i>et al.</i> ⁴⁷ , 2013	Rats	5×10^6 syngeneic MSCs, the minor mismatched MSCs and the major mismatched MSCs	8 weeks	The regenerated meniscus was different from the normal meniscus from the viewpoints of contour, cellularity, matrix staining, and type II collagen immunostaining.
Hong <i>et al.</i> ⁴⁸ , 2010	Rabbits	2×10^6 human bone marrow MSCs	12 weeks	MSCs appeared to enhance regeneration of the meniscus, although the MSCs observed in the meniscus were too few to account for the regeneration.

by transforming growth factor- β 1 (TGF- β 1) into chondrocytes rarely aggregated at the fibrocartilage surface. This could be due to the fact that TGF- β 1 induces stem cells to differentiate into hyaline cartilage. Al Faqeh *et al.* injected stem cells cultured in chondrogenic media and basal media into the joint cavity of sheep with osteoarthritis (model established by resecting the medial meniscus and the anterior cruciate ligament)²². They showed that, regardless of the induction, histological repair of the articular cartilage was observed in all the sheep injected with MSCs, and the joint was covered by cartilage tissue with similar thickness and quality compared to that in normal knee cartilage tissue.

However, when MSCs cultured in chondrogenic media were injected, they differentiated into fibrous cartilage tissues that covered the defects, whereas MSCs cultured in the basal media only formed scar tissue.

Adipose MSCs in Meniscus Repair

In an animal study, 40 Japanese rabbits have been divided into two groups, and 20 rabbits in the experimental group had an injury of 1.5 mm in diameter at the anterior horn of the meniscus⁴⁰. A cylindrical complex made up of allogeneic adipose MSCs (derived from the subscapular fat pad of rabbits) and steroid matrix was transplanted at the defects. The

control group was left untreated after injury. Meniscal defects in the experimental group were significantly smaller after 4 weeks, while there was no change in the control group. All the rabbits were killed 12 weeks after modeling, and the defects in both groups generally disappeared. However, the morphology of the regenerated meniscus in the experimental group was more similar to that of the normal meniscus. Histological analysis showed that the experimental group was positive for Safranin-O, type I collagen, and DiI-labeled HE staining, whereas the control group was negative for all these tests. These results showed that, histologically, the regenerated meniscus in the experimental group was more similar to normal meniscus fibrous cartilage, but in the control group, it was more similar to scar tissue.

A full-thickness cartilage defect rabbit model with a height of more than 5 mm has been established by Ishihara *et al.*⁴¹. Three-dimensional complexes containing bone marrow MSCs were transplanted to the defect, and it was found that uninduced MSCs differentiated into bone and cartilage simultaneously after 52 weeks of observation. Also, a clear boundary was found between the bone and cartilage. Thus, this technique might be effective for clinical treatment of bone and cartilage injuries. Neither Tatsuhiro nor Ishihara used a common absorbable scaffold as the carrier for MSCs. They developed a novel method for fusing spheroids or cylinders derived from BMSCs to make a large construct without using a scaffold. It has been shown that cylindrical or spherical structures are more suitable for the internal microenvironment of the meniscus. MSCs simultaneously forming into a spherical structure can also increase the possibility of cell survival⁴². Degradable complexes can reduce the interference of scaffolds on the meniscal microenvironment, cell differentiation, and physiological characteristics of signal factors, such as cytokines⁴³.

Studies have also compared the effect of stem cell treatment to meniscus suturing. Dutton *et al.* suggested that meniscus and cartilage morphology and repair ability, as well as load bearing in pigs, are similar to humans⁴⁴. Thus, the team assigned 28 pigs with radial meniscus tears into three groups: group 1 was the control, group 2 was given meniscus stitching with suture and protein glue, and group 3 was given joint cavity injection of simple MSCs. Groups 2 and 3 had improved treatment outcomes compared to those in the control group, and group 3 showed better meniscus healing but insignificant mechanical improvement when compared with group 2. Zellner *et al.* made a 4-mm longitudinal tear at the lateral meniscus of New Zealand rabbits and divided them into five groups based on the following treatments: 5–0 suture group, hyaluronic acid injection group, platelet-rich plasma injection group, autologous MSCs-hyaluronic acid-collagen complex implantation group, and the blank control²¹. Suture augmentation is an important research field in the application of MSCs. The stem cells could be the repair cells themselves and differentiate into fibrochondrocytes to promote meniscal healing by synthesizing. The developed repair tissue was meniscus-like and showed biomechanical

properties that can withstand physiological compressive and tensile forces toward the menisci.

Using biological methods to induce differentiation of adipose MSCs may not gain satisfactory results⁴⁵. Thus, Meier *et al.* used a custom-built device to simulate constant cyclical and uniaxial strain on cells. They showed that, under mechanical strain, adipose MSCs showed simultaneous fibrogenesis (increased expression of type I collagen and versican) and chondrogenesis (increased expression of type II collagen, Sox 9, and aggrecan). They also show that mechanical stimulation promotes fibrogenesis prior to chondrogenesis, indicating that stretching stress may be an important factor for fibrogenesis. Since simple mechanical stimulation failed to induce protein expression, both mechanical and biochemical stimulations may be required to induce cell differentiation. This may explain why, in the above experiments, the mechanical properties of some regenerated menisci could not be restored to the previous levels. Although most animals were allowed unlimited activity, knee surgery would inevitably lead to changes in joint stress. The simulation of normal joint stress of menisci in animal experiments has become a new problem for scientists to solve.

Human Meniscus Progenitor Cells in Meniscus Repair

Synovium, bone marrow, or adipose tissue in some small animals cannot provide enough MSCs, which poses a limitation. Since MSCs have low immunogenicity, some studies have suggested the use of purified human MSCs for animal experiments. Shen *et al.* built a mice meniscus injury model and injected human meniscus progenitor cells into the defects⁴⁶. The study not only confirmed the effectiveness of the treatment to promote meniscus regeneration but also identified a regulatory pathway by which the progenitor cells decreased the expression of both type I and type X collagen and increased the expression of type II collagen. These findings indicate that progenitor cells have the potential to prevent osteoarthritis and repair hyaline cartilage.

The use of non-homologous MSCs significantly compensated for the disadvantage that small animals, such as mice and rabbits, only provide a shortage of the cells⁴⁶, but some studies questioned if non-homologous MSCs could trigger autoimmune attacks. Okuro *et al.* performed syngeneic, minor, and major mismatched transplantation of synovial MSCs into the joint cavity of rats with meniscal defects and found that the repair of defects in groups injected with syngeneic and minor mismatched MSCs was superior to the major mismatched group⁴⁷. Huge amounts of macrophages and CD8 T cells were aggregated around the defects in the latter. However, Hong *et al.* used human bone marrow MSCs to treat rabbit meniscus injury and found no significant difference between the control and experimental groups⁴⁸.

Currently, artificial induction of MSCs has entered the gene level. The CRISPR/Cas9 system is one of the hottest topics discussed lately due to its robustness and effectiveness in genome editing. The technology has been widely used in

TABLE 2 Human experiments

Time	Research type	Inclusion criteria	Injection	Observation time and evaluation indicators	Outcome
Pak <i>et al.</i> ⁵⁷ , 2013	Case reports	32-year-old female	Mixed injection of platelet-rich plasma, calcium chloride, and low-dose autologous adipose MSCs (14 days after op); Platelet-rich plasma and calcium chloride (28 days after op)	Visual analogue scale (VAS) score and MRI (3 months after op)	The patient's symptoms improved, and repeated MRI showed almost complete disappearance of the torn meniscus
Vangsnæs <i>et al.</i> ⁵⁸ , 2012	Multicenter, randomized, double-blind control study	55 subjects with medial meniscus damage	Group A: bone marrow MSCs (5×10^7), human serum albumin, sodium hyaluronate, and plasma; Group B: bone marrow MSCs (1.5×10^8); Group C: sodium hyaluronate	VAS score and MRI (12 months after op)	A higher proportion of those with osteoarthritic changes experienced a reduction in pain following the treatment with mesenchymal stem cells.

life science research, including microbial, plant, animal, and human cell studies^{49–51}. CRISPR/Cas9 genome editing technology can also be used to prompt a particular phenotype in stem cell-derived target cells. With the advent of CRISPR/Cas9 technology, efficient and robust genome engineering of stem cells has raised the possibilities for more meaningful disease modeling in the relevant molecular, cellular, and anatomical contexts. CRISPR/Cas9 technology can be employed to knock-in tissue-specific selectable markers to track stem cell differentiation and reporters without disrupting the expression and function of targeted genes. In addition to real-time tracking of cell lineage and fate, tissue-specific reporters are also useful in the selection of specific cell populations during stem cell differentiation⁴⁹. It was found that only 11 genes had overlapping expression for hyaline cartilage and fibrous cartilage⁵². Only nerves and bone marrow, of all interstitial tissues, can be used as cell markers for chondrocyte differentiation and express similar gene sequences. Expression of specific genes may influence the direction of stem cell differentiation. Yi *et al.*⁵³ found that DI-NPs are good tracers for gene delivery and could potentially be used for imaging and tracing using various tools *in vitro* and *in vivo*, and it was also a good gene delivery carrier and induces chondrogenic differentiation of hMSCs. This is particularly meaningful for the meniscus, because it can simultaneously develop two morphologies, hyaline cartilage and fibrous cartilage. TGF- β 1 or insulin-like growth factor-1 (IGF-1) has been previously reported to modify gene expression in bone marrow MSCs and thus repair huge meniscus injuries *in vitro*^{54,55}. Although modification at the gene level makes the differentiation of MSCs more controllable, more evidence is needed. Also, uncontrolled expression of TGF- β 1 might result in significant joint fiber hyperplasia or serious systemic damage⁵⁵.

In fact, MSCs will lose a portion of their functions after isolation and cultivation *in vitro* as the surrounding

microenvironments are changed and not the same as the environment *in vivo*. Preconditioning of MSCs by hypoxia, pharmacological agents, chemical agents, trophic factors, cytokines, and physical factors prior to their application is capable of initiating survival signaling to counter the rigorous harsh microenvironment for the application of MSC transplantation⁵⁶.

Human Experiments

There have been only two clinical experiments of MSCs for meniscus repair (Table 2). One was a case report of a 32-year-old female, and the other was a multicenter, randomized, double-blind control study, which recruited 55 subjects with medial meniscus damage.

Autologous Adipose MSCs in Meniscus Repair

A 32-year-old female who had a grade 2 tear in her medial meniscus and had received treatment with autologous adipose MSCs has been reported⁵⁷. Joint cavity injection was performed on the day of liposuction and 3 days and 7 days after surgery, and another dose of PRP with CaCl₂ and hyaluronic acid (1 mL) was injected in the same fashion as the first day. A mixed injection of platelet-rich plasma, calcium chloride, and low-dose dexamethasone was performed 14 days after the surgery. Injection of platelet-rich plasma and calcium chloride was performed 28 days after the surgery. After 3 months of follow-up, the patient reported significantly alleviated pain. Her visual analogue scale (VAS) score was significantly lower, and MRI showed “almost fully repaired” meniscus. The team combined multiple agents for the treatment, and the conclusion was based on the premise that “platelet-rich plasma and hyaluronic acid do not improve the healing of the meniscus.” However, injection of dexamethasone into the cavity after surgery would influence VAS scoring, and thus using MRI to evaluate the level of

meniscus injury makes this study lacking and in need of improvement.

Bone Marrow MSCs in Meniscus Repair

A multicenter, randomized, double-blind control study has been conducted⁵⁸. A total of 55 subjects with medial meniscus damage were recruited from seven medical centres and randomly assigned into three groups. Group A received a cavity injection of bone marrow MSCs complex containing 5×10^7 bone marrow MSCs, human serum albumin, sodium hyaluronate, and plasma; group B received a cavity injection of bone marrow MSCs complex containing 1.5×10^8 MSCs; and group C received a cavity injection of sodium hyaluronate as control 7 days after the meniscus resection. Bone marrow MSCs were from the bone marrow bank. Meniscal volume was analyzed by semi-quantitative MRI 12 months after the surgery, and a growth greater than 15% was considered positive. The results showed that 24% of patients in group A and 6% of patients in B showed positive growth, whereas no patients had such growth in group C. This indicated that cavity injection of bone marrow MSCs could promote meniscal regeneration. In addition, patients who also had cartilage degeneration reported significantly alleviated pain after treatment with MSCs; the symptoms of patients in the control group were not significantly relieved. This is regarded as level-1 evidence of evidence-based medicine. Previous *in vitro* studies have confirmed that simply injecting human serum albumin, sodium hyaluronate, or plasma failed to promote meniscal healing, but cytokines contained in human serum albumin and plasma may induce the differentiation of bone marrow MSCs⁵⁹. Thus, there is a need to eliminate certain interference factors to evaluate if bone marrow MSCs can improve the need for meniscal regeneration.

Discussion

It is undeniable that the ability of animals to repair is different from humans, and meniscus injuries in some animals could be completely healed without any treatment in due course of time⁶⁰. Thus, if a gene modification could lead to an animal model that has a higher resemblance to pathological changes in humans, it could become an important research direction.

At present, although arthroscopic partial meniscectomy is the main method for the treatment of meniscus injury, tissue engineering and mesenchymal stem cells *in vivo* treatment of meniscus injury as a new technique has also begun a preliminary clinical exploration⁶¹. But there are ethical limitations to human experiments. This makes the progress in human experiments very slow. In addition, since different areas of the meniscus possess different healing abilities, the selection of patients is more difficult in human experiments.

In conclusion, principles for MSCs to repair meniscus injuries include the following: (i) MSCs can directly differentiate into fibrous chondrocytes; (ii) MSCs can provide

massive amounts of cytokines and signal factors by themselves that enhance cellular viability and cellular proliferation and reduce cell apoptosis; and (iii) MSCs can migrate to the site of injury to extensive proliferative and anti-inflammatory. Several studies have shown that MSCs derived from a variety of connective tissues demonstrated remarkable efficacy in promoting meniscus regeneration and repair⁶². It has been found that, in addition to acting as seed cells that possess the ability of multi-directional differentiation, MSCs can secrete cytokines or growth factors by autocrine or paracrine signaling⁶³. Under the regulation of stem cells, the level of cytokines, including TGF- β , IGF-1, bone morphogenetic protein (BMP), and fibroblast growth factor (FGF) increased several hundred-fold. Meanwhile, MSCs can suppress damage-induced inflammation by regulating multiple anti-inflammatory cytokines, such as interleukin (IL)-10, TGF- β , and prostaglandin E2 by paracrine regulation, thereby providing the proper microenvironment for meniscus healing⁶⁴. MSCs can generate synovial tissue during meniscal repair, which provides more nutrition for the meniscus. This was proposed by Nakagawa⁶⁵ who injected synovial MSCs in to minipigs with meniscus injury and found that synovial MSCs promoted meniscal healing.

Currently, a large number of preclinical studies have shown that *in vivo* injection of MSCs to treat meniscus injury is a safe, reliable, and efficient method to repair meniscus injury. Although human clinical research has been carried out, it still has great limitations. First, human clinical research involves ethical restrictions, so it is difficult to carry out it on a large scale, which leads to the slow progress of the research. Second, these few clinical studies lack a good control group as a reference, lack long-term follow-up, and lack an end point of objective results after *in vivo* injection. Third, the complexity of the human meniscus tissue itself, partial vascularity, multiple cell types in the tissue, and interspecific variation of the meniscus may add some difficulties to the experiment. Fourth, compared with repairing cartilage, meniscus repaired by MSCs lack of postoperative histological examination, imaging examination, and arthroscopic examination left researchers unable to identify the type of newborn tissue, in addition to a lack of reliable experimental results. Lastly, there is no consensus on the ideal cell source and scaffold for the treatment of meniscus injury by *in vivo* injection of MSCs⁶⁶.

The treatment of meniscus repair and regeneration is very limited as a new method, and stem cells promote meniscus regeneration in preclinical research and human preliminary research. We expect that, in the near future, *in vivo* injection of stem cells to promote meniscus repair can be used as a new treatment model in clinical treatment.

Conclusion

Due to the special anatomical features of the meniscus, conservative or surgical treatment can hardly achieve complete physiological and histological repair. Cytology treatment is a very promising field for post-damage

meniscus repair. Many animal experiments and a few human experiments indicate that MSCs can promote meniscal regeneration, but the lack of human evidence and defects of animal experiments have left many problems unsolved. As for the animal experiments, most models of meniscus injury are too simple, which can hardly simulate the complexity of actual meniscal tears,

and since the follow-up often lasts for only 4–12 weeks, long-term results could not be observed. Lastly, animal models failed to simulate the actual stress environment faced by the meniscus, so it needs to be further studied if regenerated meniscus has similar anti-stress or anti-twist features. Despite these limitations, repair of the meniscus by MSCs has great potential in clinics.

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