Stem cell therapies in tendon-bone healing

Yue Xu, Wan-Xia Zhang, Li-Na Wang, Yue-Qing Ming, Yu-Lin Li, Guo-Xin Ni

ORCID number: Yue Xu 0000-0002-6051-5415; Wan-Xia Zhang 0000-0002-2573-4321; Li-Na Wang 0000-0003-3627-5674; Yue-Qing Ming 0000-0002-4731-3798; Yu-Lin Li 0000-0003-0368-7218; Guo-Xin Ni 0000-0001-9181-8155.

Author contributions: Xu Y wrote the paper; Zhang WX, Wang LN, Ming YQ, and Li YL collected the data; Ni GX made critical revisions related to important intellectual content of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Yue Xu, Wan-Xia Zhang, Li-Na Wang, Yue-Qing Ming, Yu-Lin Li, Guo-Xin Ni, School of Sports Medicine and Rehabilitation, Beijing Sport University, Beijing 100084, China

Corresponding author: Guo-Xin Ni, MD, PhD, Chief Doctor, Professor, School of Sports Medicine and Rehabilitation, Beijing Sport University, No. 48 Shangdi Information Road, Haidian District, Beijing 100084, China. fjrehab@163.com

Abstract

Tendon-bone insertion injuries such as rotator cuff and anterior cruciate ligament injuries are currently highly common and severe. The key method of treating this kind of injury is the reconstruction operation. The success of this reconstructive process depends on the ability of the graft to incorporate into the bone. Recently, there has been substantial discussion about how to enhance the integration of tendon and bone through biological methods. Stem cells like bone marrow mesenchymal stem cells (MSCs), tendon stem/progenitor cells, synovium-derived MSCs, adipose-derived stem cells, or periosteum-derived periosteal stem cells can self-regenerate and potentially differentiate into different cell types, which have been widely used in tissue repair and regeneration. Thus, we concentrate in this review on the current circumstances of tendon-bone healing using stem cell therapy.

Key Words: Tendon; Bone; Stem cell; Anterior cruciate ligament; Rotator cuff

Core Tip: The key to tendon and ligament reconstruction is the strong integration of tendon and bone. Multiple types of stem cells have widely used to improve the tendon-bone healing. There are advantages, disadvantages, and differentiation capabilities for each type of cell. Numerous biological methods have been used, such as gene modification therapy and tissue engineering. However, the mechanism of the stem cells implanted for tendon-to-bone healing is unknown, and further research is necessary. The research on tendon-bone healing using stem cells is reviewed here.

INTRODUCTION

The incidence of tendon-bone insertion (TBI) injuries has increased with today's growing focus on sporting activities. TBI injuries are highly common in both the workplace and sports, such as rotator cuff and anterior cruciate ligament (ACL) injuries; in fact, over 30 million injuries occur worldwide each year[1]. The treatment of such orthopedic injuries includes the reconstruction of the tendon/ligament by moving a tendon graft into a bone tunnel to restore its insertion into the bone of a ruptured tendon or ligament. The success of these reconstructive procedures relies on the ability of the graft to incorporate into the bone.

Failure of tendon-to-bone remodeling can result in high rates of relapse after surgery. Tendon-bone healing also contributes to scar tissue formation, which has limited mechanical properties that make the healed tendons vulnerable to re-injury and thus have elevated re-tear rates[2,3]. The failure rates for rotator cuff repair, for example, have been reported to range from 20% to 94%[2,4], while ACL reconstruction has a failure rate of 10% to 25%[3]. In addition, inadequate tendon-bone healing can result in various secondary symptoms, including knee laxity, movement instability, damage to the meniscus and cartilage, and even early post-traumatic osteoarthritis[5, 6]. The site of the tendon graft attachment to the bone is the weakest site in the early healing phase. To protect the healing graft from undue loads, it is also important to postpone mobilization, physical therapy, range of motion exercises, and complex activities[7]. The recovery period is lengthy, preventing an early return to sports.

Various experimental models have shown that after ligament repair, the complex structure and composition of the normal insertion site are not regenerated[7-9]. The interface between the transplanted tendon and the bone tunnel normally recovers along with the fibrous scar tissue, and the configuration and composition of the native direct tendon-bone interface are not reformed during curing; thus the interface mechanically and structurally decreases[10]. The poor healing of the tendon bone interface occurs due to various causes. First, osteointegration and graft remodeling typically recover slowly. Second, if the tendon injury is considerable or if the tendon retraction is broken, the tendon is poorly regenerated after trauma and degeneration [11]. Third, the fibrocartilage area has relative avascularity and bone loss at the injury site[12].

In orthopedics and sports medicine science and clinical practice, the firm healing of a tendon graft to bone involves the healing of two inhomogeneous tissues, which thus has been considered a crucial factor for the long-term success of many reconstructive operating procedures for weakened tendons. The effective integration of tendon-bone healing remains a challenge.

Accelerating and improving tendon-to-bone healing can diminish the risk of injury and allow earlier and more aggressive rehabilitation as well as earlier recovery from work, recreation, or pre-injury activities[13]. Scholars have therefore sought biological therapies to enhance the healing of the tendons. In orthopaedic basic science studies, methods to biologically accelerate and optimize tendon-to-bone healing have been studied meticulously over the past decade. In recent years, techniques such as the use of growth factors[14], platelet-rich plasma (PRP)[15], cell-based therapies[16,17], gene transfer[18], tissue engineering[19], and numerous other methods of delivery and induction have been applied and actively developed.

Stem cells can differentiate in endogenous and exogenous factors into established cell types; they are thus becoming increasingly interesting for researchers. The purpose of this paper is to delineate the current understanding of tendon-bone healing, and to summarize the current state of stem cell therapy for tendon-bone healing.

PHYSIOLOGICAL PROCESS OF TENDON-BONE HEALING

Native structure of TBI

The native TBI is a highly specialized and organized tissue[20] and deforms as a fibrous network[21], which acts as a stress absorber during mechanical loading[22] and transmits complex mechanical loads from soft tissue to bone. In general, TBI can be divided into two categories: Indirect and direct insertions. Indirect insertions, such as the tibial insertion of the medial collateral ligament, include tendon, Sharpey's fibers, and bone. In contrast, direct insertions, such as the insertion of the ACL and rotator cuff, can be classically categorized into four zones. Benjamin *et al*[23] reported that the research of Dolgo-Saburoff in 1929 and Schneider in 1956 first showed four zones at TBI (Figure 1).
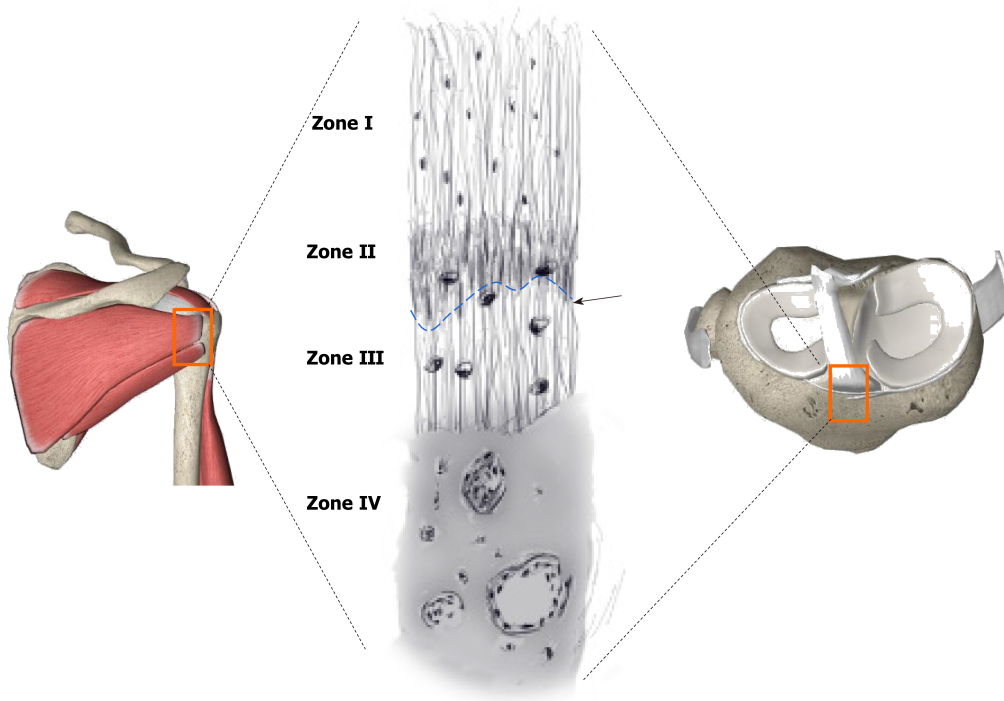
Figure 1 Structure of the tendon-bone insertion. Zone I consists of ligament. Zone II comprises nonmineralized fibrocartilage. Zone III is composed mineralized cartilage. Zone IV consists of bone. Tidemark between Zone II and Zone III (black arrow) is shown.

Zone I is a ligament that contains an extracellular matrix of proteoglycans and consists of highly aligned type I collagen[24,25], particularly of dermatian sulphate. The only cells in this region are fibroblasts. The mechanical properties are identical to the mid-substance tendon-like properties[26]. Zone II is a nonmineralized cartilage region of fibrocartilage cells aligned within the bundles of collagen. This zone primarily contains a mixture of forms of collagen II and III and the proteoglycan aggregate[27] and has a more networked organization[28]. Zone III is the region of cartilage that is mineralized. The fibrocartilage is mineralized in this area and inserted into the subchondral bone layer. Between Zones II and III, there is a distinct basophilic "blue line" or the "thread mark" between the mineralizations. The structure is reinforced by mineral deposits on the nanometer scale[29]. Here, collagen type II predominates, and both the collagen type X and aggrecan are important in specific quantities[26,27]. Zone IV is made up of bone attached to the ligament. Its matrix consists mainly of chondroitin sulfate and mainly of collagen type I mineralized in osteoblasts, osteocytes, and osteoclasts[28]. Traditionally, these areas are known to be explicitly differentiated but structurally continuous. This particular anatomy insertion point is intended to serve as a "two-layered defense system" shielding the bone from excessive shear stress and the tendon from excessive pressure or strain.

Process of tendon-bone healing

A dog tendon-bone healing model was used by Rodeo *et al*[8], who suggested that bone tendon healing should be carried out by the development of a fibrovascular interface tissue between the tendon and bone, accompanied by gradual bone ingrowth into this interface tissue. In the 24 wk after ACL reconstruction in rabbits, Schiavone Panni *et al*[30] demonstrated that the junction between tendon and bone was morphologically complete. Grana *et al*[7] then found in an ACL reconstruction model of rabbit, the strengthening of collagenous fibers in the tubes in a bone at 12 wk after the operation had been completed by intertwining of graft and connective tissue and the attachment of connective tissue to bone in tunnels. The collagenous fibers appear in an indirect inclusion of the Sharpey's fibers. In 1997, Liu *et al*[31] outlined the process of tendon-bone healing in a rabbit model. Inflammatory cells in the tendon-bone interface are present after 1 wk. Scar tissue occurred after 2 wk. The scar tissue at the interface from bone to tendon was then gradually reorganized 4 wk after the surgery into a dense connective tissue matrix, continuously with bone and tendon juxtapositions. After 6 wk, a more longitudinal reorganization of the disorganized collagen fibers was observed, and Sharpey's fibers were noted.

The histological findings concerning hamstring insertion healing of the ACL reconstruction in 12 patients were recorded by Robert *et al*[32]. The initial tendon-bone contact region was osteolyzed, with lamellar bones partially covered by an osteoid bone at a rate of 3 mo replaced with highly cellular and fibrovascular tissue. After 6 mo, the fibrous interface came into continuous contact with the lamellar bone osteoid tissue, while Sharpey-like fibers began to become evident. The quantity of Sharpey-like fibers became apparent at 10 mo, and the healing process matured. It was reported that the time needed for a mature indirect anchorage was 10 to 12 mo, which is approximately 6 to 24 wk longer than that in the animal model. But few studies have examined tendon-to-bone healing for human beings, mostly because patients have biopsied the whole grease in a small focal area, without examining the entire graft, as would be done in an animal model[33]. Second, the harvest is simple using an arthroscopic technique in the joint part of a graft, although it is truly difficult to harvest the bone-tendon intersection[34].

Lui *et al*[35] summarized the tendon graft stage a few years later for the bone tunnel cure. It can be divided into four different phases. Stage 1 is the inflammatory phase; during this stage, inflammatory and marrow-derived stem cells are invaded and recruited. An interposed fibrovascular layer of scar tissue is at the interface between the graft and tunnel. Stage 2 is the proliferative phase, which involves the proliferation and differentiation of the stem cells. Interface cells release cytokines and growth factors, resulting in blood vessels and nerves growing[36]. Stage 3 involves the process of matrix synthesis, and the new extracellular matrix is synthesized and processed with the incremental entry of the bone. Stage 4 is the phase of the matrix regeneration, wherein, the newer bone, interfacial fabric, and graft remodeling, like Sharpey's fibers of an indirect insertion, are developed with consistency between the tendon graft and the bone of collagen fibers[37,38]. A decrease in cellularity occurs at the interface. As Sharpey's fibers continuously increase over time, the mechanical strength of the interface increases gradually.

TYPES OF STEM CELLS

Stem cells have the capacity to self-regenerate, differentiate into different cell types, and harness a repair mechanism by being able to divide without limits to regenerate other cells; they are therefore currently being studied by researchers and becoming a common treatment option for musculoskeletal injuries such as ACL injuries and rotator cuff tears. For successful results of stem cell therapies, the cell source is important[39].

Numerous sources for stem cells are available and so stem cells can also be classified according to their origin tissue: Embryonic stem cells, fetal stem cells, umbilical cord stem cells, and adult stem cells. One study has shown that human placenta-derived mesenchymal stem cells (MSCs) with strong proliferative ability have rich sources and can remarkably promote tendon-bone healing in a rat model after cell transplantation [40], but the ethical and legal issues arising from human placenta-derived MSCs, making it difficult to perform more trials. It still needs more evidence to confirm the efficiency of placenta-derived MSCs on tendon-bone healing.

There are currently two principal types of clinically promising adult stem cells: Hematopoietic stem cells and MSCs. Several recent studies have shown that MSCs are obtainable from synovium[41], periosteum[42], and adipose tissues[43], which have been widely used in tendon-to-bone repair. Each source has its advantages, inconveniences, and capacity for differentiation. Bone marrow MSCs (BMSCs) can be collected from the anterior iliac crest, posterior superior iliac crest, tibial tubercle, or sternum. The harvesting method is invasive and requires local anesthesia, which can cause additional pain in patients. The study suggested that the *in vitro* expansion possibilities of BMSCs were limited[44]. The tendon stem/progenitor cells (TSPCs), which have proliferated more colongenically than BMSCs[45], exhibited higher colongenicity. In addition, TSPCs have indicated higher tenogenic markers, such as scleraxis (Scx) and tenomodulin (Tnmd)[45]. The TSPCs, such as those in tendons of Achilles, patella tendons, supraspinatus seins, or hamstring tendons, may be harvested from waste tendon tissue during tendon and ligament surgery. However, sufficient TSPCs are required in the *in vitro* cell culture. Recently, more and more studies have uncovered the potential both for proliferation and for chondrogenesis in synovium-derived MSCs (SMSCs) rather than in BMSCs[46,47]. However, SMSCs require secondary surgery from the knee joints and shoulder. It requires a long duration of *in vitro* expansion to achieve sufficient stem cells. Adipose-derived stem cells (ADSCs)

can be obtained in the hips and thighs, or around incisions, from the subcutaneous adipose tissue. Studies have demonstrated that adipose has significantly more MSCs than bone marrow, and the yield of ADSCs is higher than that of BMSCs. Periosteum-derived periosteal stem cells (PSCs) can be harvested from the tibia or the humerus inferior to the tip of the greater tuberosity. An analysis found that the osteogenic, chondrogenic, and adipogenic capacities of rat PSCs were greater than those of BMSCs [48]. Table 1 indicates various differentiation capacities among MSCs.

BMSCS

The use of biological agents to improve tendon bone healing is becoming increasingly relevant. Studies have proven the potential for better tendon-bone healing for a decade using bone morphogenic protein (BMP)-2[49] and a mixture of bone growth proteins [14]. BMSCs can be collected from the pelvic, femurs, tibias, and humeri. BMSCs are pluripotential cells that have been extensively studied in the treatment of tissue injury [50,51].

Pre-clinical studies

Lim *et al*[52] and Ouyang *et al*[53] used BMSCs for the first time in the study of tendon-bone healing. Lim *et al*[52] investigated the impact of BMSCs grafting of a rabbit ACL on the rate and consistency of graft osteointegration. After 2 wk, the BMSCs group had large areas of cartilage cells at the tendon-bone junction. The BMSCs group had a mature cartilage zone after 8 wk, which stained strongly for collagen type II, blending a mature cartilage zone, and increased failure load and stiffness. They concluded that the coating of tendon grafts with BMSCs contributes to a cured cartilage zone close to regular ACL inserts instead of collagen fibers and scar tissues. This also helps in the treatment of the collagen fibers. A few years later, Lim and his colleagues[54] studied a rabbit ACL reconstruction model and found similar results, the effect of coating allografts with BMSCs on the consistency and rates of the osteointegration of the allograft tendon and bone interface. In 2019, Hur *et al*[55] investigated the effects of BMSCs on tunnel widening in a rabbit ACL reconstruction model. The BMSCs group had a lower mean for the enlargement of the femoral tunnel and an extension of a tibial tunnel to the oblique sagittal image. Hypercellular architecture and compact collagen deposition were present in the BMSCs group. They showed that BMSCs appear to reduce the expansion of the tunnel in the reconstruction of ACL.

The effectiveness of using a large number of BMSCs to boost tendon-to-bone healing in a rabbit tendon-bone healing model was evaluated by Ouyang *et al*[53]. The BMSCs group exhibited more perpendicular forming of collagens and an increased growth in cartilage-like cells, which was indicated by positive collagen type-II immuno-staining of the tendon-bone interface. They argued that BMSCs could enhance the insertion of tendon healing to the bone through the formation of fibrocartilaginous attachment at an early stage. Seven years later, Li *et al*[56] investigated the effects of BMSCs on a model of rat tendon-bone healing. In the BMSCs group, at 4 and 8 wk after surgery, the maximum biomechanical production strength was significantly higher.

Some studies then compared the effects of various cells. In a rat model of degenerative enthesis reparation, Nourissat *et al*[57] assessed the impact of depositing BMSCs or chondrocytes. They developed a model based on the destruction of the Achilles tendons enthesis and found that rats with BMSC injections were arranged into the column of chondrocytes 45 d after surgery in a native enthesis. Kida *et al*[16] analyzed the kinetics of BMSCs with bone marrow chimeric rats, which expressed green fluorescent protein (GFP) during the rotator cuff repair. An additional drilling procedure was performed at the greater tuberosity. They found that more GFP-positive cells and a higher rate of ultimate force-to-failure in the drilling group. After 3 years, Degen *et al*[58] assessed the capacity of human BMSCs to augment the healing of an acute small-to-medium-sized rotator cuff repair in a rat model. At the repair site, BMSCs increased the development of fibrocartilage, strengthened collagen orientation, and increased load failure and stiffness over 2 wk. In the histological and biomechanical findings, there were nevertheless no major variations in 4 wk. Lu *et al*[59] then compared the effects on tendon-to-bone healing of the fresh autologous bone marrow and BMSCs with a novel rat model. The bone marrow group showed a relatively well-organized and dense connecting tissue interface with an enhanced collagen fiber orientation, less M1 macrophages and more M2 interface macrophages. Biomechanical tests indicate that the bone marrow group exhibits considerably higher stiffness. They demonstrated that local delivery of fresh autologous bone marrow better improves

Table 1 Summary of differentiation capacities between different mesenchymal stem cell types

MSC type	Clinical extraction methods	Osteogenic	Chondrogenic	Adipogenic
BMSCs	From the anterior iliac crest, posterior superior iliac crest, tibial tubercle, or sternum	++	++	+
SMSCs	From the knee joints and shoulder during surgery	++	++++	+++
ADSCs	Liposuction at subcutaneous adipose tissue	+	+	+++
PSCs	Stripped from the tibia	+++	+++	++

MSC: Mesenchymal stem cell; BMSCs: Bone marrow mesenchymal stem cells; SMSCs: Synovium-derived mesenchymal stem cells; ADSCs: Adipose-derived stem cells; PSCs: Periosteum-derived periosteal stem cell.

tendon-to-bone healing more than BMSCs due to inflammatory modulations.

On the contrary, Gulotta *et al*[60] determined the effect of BMSCs in a rat rotator cuff healing model. Histological and biomechanical evaluations between groups did not vary at any given time. The study concluded that adding BMSCs to the insertion site of the healing rotator cuff did not change the site's structure, composition, or strength. Similarly, a study by Kanazawa *et al*[61] concluded that the use of BMSCs alone in a reconstruction model of rabbit ACL with no tibial bone tunnel was unable to enhance the tendon-to-bone healing to a sufficient extent (Table 2).

Stimulating factors

Above all, the results of using BMSCs in tendon-bone healing are inconsistent. Many researchers believe that different stimulating factors with BMSCs may enhance tendon-bone healing. In 2016, Teng *et al*[62] investigated the potency of PRP combined with BMSCs to advance tendon-bone healing in a rabbit ACL reconstruction model. A more mature interface with aligned chondrocytes and a higher failure load was observed in the BMSC + PRP group. They concluded that the combination of PRP and BMSCs promoted tendon-bone healing. Han *et al*[63] found similar histological and biomechanical findings regarding the treatment of tendon-bone injuries after rotator cuff injuries a couple of years later. They proved that the combination of BMSCs and PRP synergistically stimulates tendon bone healing through stronger signals associated with angiogenesis, bone formation, and *in situ* tendon generation. Setiawati *et al*[64] then tested whether injecting BMSCs and vascular endothelial growth factor (VEGF) into the bone tunnel of rabbits after ACL reconstruction would improve the early healing process of a tendon graft. They found a higher mean collagen type III score, a higher value for the final tensile strength, as well as improved signal intensity for magnetic resonance imaging (MRI) in the group BMSCs + VEGF. They also demonstrated that BMSC and VEGF injections of intra-tunnels improved the graft tunnel healing after ACL reconstruction.

Tissue engineering

Stem cell tissue engineering is becoming more commonly used in tendon-bone cure due to the rapid advancement of tissue technology. In 2018, Thangarajah *et al*[65] investigated the potential of a demineralized bone matrix (DBM) improved with BMSCs, when used in a degenerative rotator cuff tear model, to enhance tendon bone healing. A DBM consists of a network of collagen fibers that provide a sustained release of growth factors such as BMPs[66]. All groups showed that the tendon-bone gap closure was observed with fibrocartilaginous entheses and the DBM + BMSCs group reached not significantly lower than non-operative checks in bone mineral densities. They concluded that the biological increase in the DBM and MSC rotator cuff repair will minimize the incidence of re-tear. In a canine non-weight-bearing model of acute, full-thickness tendon injury, Liu *et al*[67] examined the effects of an engineered tendon-fibrocartilage-bone composite (TFBC) combined with BMSCs sheet on rotator cuff healing. The TFBC includes native fibrocartilage enthesis focused on more durable tendon-to-tendon and bone-to-bone homogeneous tissue cure, as well as a multilayered cell seeding structure close to the collagen-cell composition of the native tendon[68]. The TFBC + BMSCs group had increased formation of new fibrocartilage and collagen fiber organization at the interface of the tendon bone and the ultimate failure and stress. They evidenced that the application of an engineered TFBC and BMSCs can enhance rotator cuff healing.

Table 2 Summary of pre-clinical studies using bone marrow mesenchymal stem cells

Ref.	Animal/model	Cell/cell number	Method of delivery	Control	Outcome measurement/timeline
Lim *et al*[52], 2004	Rabbit/Bilateral ACL reconstructions using hamstring tendon autografts	BMSC/3-4 × 10^6	Grafts were coated with BMSCs in a fibrin glue carrier	ACL reconstruction without BMSCs	Histology, biomechanics/2, 4, and 8 wk
Ouyang *et al* [53], 2004	Rabbit/The hallucis longus tendons were translated into calcaneal bone tunnels	BMSCs/ × 10^7	BMSCs immobilized in fibrin glue were injected in the bone tunnel	Translation without BMSCs	Histology, immunohistochemistry/2, 4, and 6 wk
Soon *et al*[54], 2007	Rabbit/Bilateral ACL reconstructions using Achilles tendon allografts	BMSCs/4 × 10^6	Grafts were coated with BMSCs in a fibrin glue carrier	ACL reconstruction without BMSCs	Histology, biomechanics/2, 4, and 8 wk
Gulotta *et al* [60], 2009	Rat/Unilateral detachment and repair of the supraspinatus tendon	BMSC/ × 10^6	BMSCs were pelleted down and mixed with fibrin sealant for implantation	Rotator cuff repair without BMSCs	Histology, biomechanics, cell tracking/2, 4 wk
Nourissat *et al* [57], 2010	Rat/Destructing the enthesis of the Achilles' tendon and attaching the tendon back to the calcaneum	BMSCs/4 × 10^6, chondrocytes/4 × 10^6	Cells were injected at the site of repair	Defect without cells	Histology, immunohistochemistry, biomechanics/15, 30, and 45 d
Li *et al*[56], 2011	Rat/The isolated flexor digitorum longus tendon were translated into tibia bone tunnels	BMSCs/5 × 10^6	BMSCs were injected into the tendon-bone interface	Translation without BMSCs	Biomechanics, cell tracking/2, 4, and 8 wk
Kida *et al*[16], 2013	Rat/Bilateral detachment and repair of the supraspinatus tendon	BMSCs/-	Drilling into the bone marrow was performed in the greater tuberosity	Rotator cuff repair without drilling	Histology, biomechanics, cell tracking/2, 4, and 8 wk
Kanazawa *et al*[61], 2014	Rabbit/Bilateral ACL reconstruction without a tibial bone tunnel using semitendinosus tendon autografts	BMSCs/ × 10^6	BMSCs with the collagen sponge or a fibrin sealant were transplanted between the bottom of the grafted tendon and the bone pit of the tibia	ACL reconstruction without BMSCs	Histology, immunohistochemistry/4 and 8 wk
Teng *et al*[62], 2016	Rabbit/Unilateral ACL reconstructions using hamstring tendon autografts	BMSCs/ × 10^7	Graft wrapped with PRP and BMSCs in fibrin glue and secured to the periosteum by surgical	ACL reconstruction without BMSCs	Histology, biomechanics, RT-PCR, micro-CT scan/4 and 8 wk
Degen *et al* [58], 2016	Rat/Unilateral detachment and transosseous repair of the supraspinatus tendon	Human BMSCs/ 10^6	BMSCs placed at the tendon-bone interface with fibrin glue	Rotator cuff repair without BMSCs	Histology, biomechanics/2 and 4 wk
Setiawati *et al* [64], 2017	Rabbit/Unilateral ACL reconstructions using semitendinosus tendon autografts	Rabbit BMSCs/2 × 10^6 with 100 μg VEGF	BMSCs were injected into the bone tunnel	ACL reconstruction without BMSCs	Biomechanics, immunohistochemistry, MRI/3 and 6 wk
Thangarajah *et al*[65], 2018	Rat/Unilateral detachment and transosseous repair of the supraspinatus tendon	BMSCs/10^6	BMSCs + DBM placed on the decorticated humeral head with fibrin glue	Rotator cuff repair without DBM	Histology, cell tracking, pqCT/6 wk
Lu *et al*[59], 2019	Rat/The isolated flexor digitorum longus tendon were translated into tibia bone tunnels	BMSCs/10^6	BMSCs were injected into the tendon-bone interface	Translation without BMSCs	Histology, immunohistochemistry, biomechanics/2 and 6 wk
Liu *et al*[67], 2019	Dog/Unilateral full-thickness infraspinatus tendon injury was created	BMSCs/10^6	Detached tendon was sandwiched between the two tendon layers of TFBC and one BMSCs sheet seeded on the tendon interfaces	Rotator cuff repair without BMSCs	Histology, biomechanics/6 wk
Hur *et al*[55], 2019	Rabbit/Unilateral ACL reconstructions using extensor digitorum longi tendon autografts	BMSCs/1.0 -1.5 × 10^7	BMSCs embedded in fibrin glue was injected to the end of graft	ACL reconstruction without BMSCs	Histology, micro-CT/12 wk
Han *et al*[63], 2019	Rat/Unilateral detachment and repair of the supraspinatus tendon	BMSCs/-	-	Rotator cuff repair without BMSCs	Histology, biomechanics, RT-PCR/4 and 8 wk
Huang *et al* [71], 2020	Rat/Unilateral detachment and reconstruction of the	BMSC-Exos/200 μg	BMSC-Exos were injected into the tail vein	Rotator cuff repair with PBS	Histology, immunohistochemistry,

	supraspinatus tendon				biomechanics, RT-PCR, micro-CT/4 and 8 wk
Shi *et al*[70], 2020	Mice/Destructing the enthesis of the Achilles' tendon and attaching the tendon back to the calcaneum	BMSC-Exos/-	Exos mixed with hydrogel and implantation in the mice	Defect without Exos	Histology, immunohistochemistry, biomechanics, RT-PCR/7, 14, and 30 d

BMSC: Bone marrow mesenchymal stem cell; ACL: Anterior cruciate ligament; MRI: Magnetic resonance imaging; pqCT: Peripheral quantitative computed tomography; DBM: Demineralized bone matrix; RT-PCR: Reverse transcription polymerase chain reaction; RT-qPCR: Reverse transcription quantitative polymerase chain reaction; TFBC: Tendon-fibrocartilage-bone composite; Exos: Exosomes; PBS: Phosphate-buffered saline.

Exosomes

As major mediators of intercellular communication, exosomes emerge to regulate a range of biological processes by means of the transmission of biological signs between cells. Exosomes are highly biomass-free and can remove the barrier of the tissue. Exosomes are beginning to be investigated in relation to diseases, including cancer, infectious disorders, and neurodegenerative disorders[69]. But in the process of tendon-bone healing, there is a lack of research on the mechanism of exosomes. In 2020, Shi *et al*[70] investigated the effects of BMSC-derived exosomes (BMSC-Exos) on tendon-bone healing. In the BMSC-Exos group, more fibrocartilage, higher maximum force, higher intensity, higher elastic modulus, higher M2 macrophage numbers, and more anti-inflammatory and chondrogenic factors were observed. They demonstrated that by enhancing M2 macrophage polarization in tendon-to-bone healing, BMSC-Exos promote the formation of fibrocartilage. Subsequently, Huang *et al*[71] explored the role of BMSC-Exos in a rat rotator cuff reconstruction model. BMSC-Exos stimulated angiogenesis across the interface of the tendon bone and prevented M1 macrophages from releasing associated inflammatory factors. After rotator cuff repair, BMSC-Exos improved the breaking load and stiffness. They concluded that BMSC-Exos facilitate tendon-bone healing by encouraging angiogenesis and suppressing inflammation *via* the VEGF and Hippo signaling pathways.

Gene therapy

Many scientists believed that BMSCs alone are not adequate to improve tendon-bone healing, and they hypothesized that BMSCs need a signal to increase their effectiveness, such as a growth factor or transcription factor[72]. Hence, in tendon-bone healing studies, gene-modified BMSCs are commonly used.

In 2010, Gulotta *et al*[72] examined whether BMSCs transduced with adenoviral membrane type 1 matrix metalloproteinase (Ad-MT1-MMP) could enhance healing following the repair of rotator cuffs when applied to the tendon-to-bone insertion site. MT1-MMP is a membrane-bound matrix of metalloproteinase, which plays a role in the embryological formation of musculoskeletal tissues[73,74]. MT1-MMP is induced by promoting the dissolution of the anlagen cartilage and the production of mineralizing cartilage[73] to guide the process of endochondral ossification. In any outcome variable, there were no variations after 2 wk. The Ad-MT1-MMP group had more fibrocartilage, increased ultimate failure load, ultimate failure tension, and higher stiffness values after 4 wk. It was concluded that genetically modified BMSCs would stimulate rotary cuff healing by increasing the amount of fibrocartilage at insertion and by improving bio-mechanical strength by overexpressing developmental gene *MT1-MMP* after 4 wk. Then they applied BMSCs transduced with adenoviral-mediated Scx, a basic helix-loop-helix transcription factor that is believed to direct tendon development during embryogenesis[75], in a rat rotator cuff repair model[76]; they found similar results. However, they found negative results when using BMSCs transfected with BMP-13 in a rat rotator cuff model in the same year[77]. They concluded that applications to overexpress MT1-MMP or Scx of genetically modified MSCs can enhance the early treatment of rotator cuff.

Several years later, Dong *et al*[78] investigated the efficacy of a rabbit reconstruction model of BMSCs with BMP-2 for tendon-bone healing. Higher maximum loading and stiffness and increased proliferation of cartilage-like cells and fibrocartilage-like tissue were observed for the BMSCs + Lv-BMP-2 group. After 5 years, Wang *et al*[79] explored the role of transforming growth factor-beta (TGF-β) in tendon-bone healing after ACL reconstruction. BMSCs were infected with TGF-β. There were more fibroblasts, a tighter tendon-bone interface, and an increasing number of cells and fibrochondrocytes close to chondrocytes and other collagen-fibers, in the overexpression group. Due to maximum load and stiffness, the bone tunnel and bone tunnel

margin computed tomography values in the overexpression group were substantially up-regulated. It was shown to promote tendon-to-bone healing by up-regulating TGF-β expression in BMSCs after ACL reconstruction by adjusting the signal pathway TGF-β/MAPK. The effect of platelet-derived growth factor subunit B (PDGF-B) on tendon-bone healing after rotator cuff injury repair by modifying BMSCs was then investigated by Wang *et al*[80]. BMSCs were transfected with recombinant lentiviral vector-encoded PDGF-B shRNA, overexpressed Pc-DNA PDGF-B, and irrelevant plasmid. They found that PDGF-B could promote viability, osteogenic differentiation, and adipogenic differentiation. The ultimate load and stiffness of the tendon tissue can be improved by PDGF-B. They evidenced that overexpressed PDGF-B could act to enhance tendon-bone healing after rotator cuff injury repair (Table 3).

Clinical studies

The clinical use of BMSCs was discussed in many orthopedic fields as a means to improve tissue regeneration. Recently, researchers have demonstrated that BMSCs cross holes drilled in the humerus footprint, penetrated the repaired rotator cuff, and contributed to the healing of the postsurgical rotator cuff[16]. Multiple channeling technology is a simple and straightforward procedure which allows BMSCs to travel from the cavity of bone marrow to the repair site of the rotator cuff tendon in the greater tuberosity of the proximal humerus[81].

The impact of BMSCs on the clinical and structural findings regarding arthroscopic rotator cuff repair was first investigated by Jo *et al*[82]. Among 124 patients, 57 underwent multichannel arthroscopic rotator cuff repair. They followed the patients for at least 2 years. Outcome measures included the visual analog scale (VAS) for pain, range of motion (ROM), MRI, computed tomography arthrography, and commonly used functional scores such as the American Shoulder and Elbow Surgeonsscale, the Constant score, the University of California, Los Angeles (UCLA) scale, the Disabilities of the Arm, Shoulder and Hand scale, the Simple Shoulder Test, and the Shoulder Pain and Disability Index scale. In terms of VAS, ROM, pain, strength, and functional scores, there was no statistical difference between the multiple channel group and conventional group. However, the re-tear rate was 22.2% in the multiple channel group, slightly lower than that of the conventional channel group. They concluded that the extravasation of bone marrow containing MSCs from the proximal humerus to the repair site was caused by multiple channeling, and the re-tear rate decreased significantly after the repair of arthroscopic rotator cuffs. Similarly, Taniguchi *et al*[83] investigated the effect of BMSCs on cuff repair integrity after arthroscopic surface-holding (ASH) repair using multiple channeling in 111 patients. Sugaya's classification was used to evaluate cuff integrity *via* postoperative MRI. The re-tear rate for large-scale tears was significantly higher in the non-BMSCs group (28.6%) than in the BMSCs group (4.5%). They showed that the application of BMSCs to the footprint during ASH repair results in an improved integrity of the cuff repair particularly in massive tears. Among 90 patients who underwent arthroscopic rotator cuff repair involving injecting cells into the interface, Hernigou *et al*[84] assessed the efficiency of biological augmentation of rotator cuff repair with BMSCs. They used MRI and ultrasound at least 10 years later to confirm healing or re-tearing of the rotator cuff. They found that 87% of patients in the BMSCs group had intact rotator cuffs, but only 44% patients in the control group. In conclusion, during rotator cuff repair, the injection of BMSCs as a form of adjunctive therapy increased the healing rate and improved the consistency of the repaired surface, as determined by ultrasound and MRI.

In comparison, Silva *et al*[85] concluded that in 43 patients, adult non-cultivated BMSCs do not tend to accelerate graft-to-bone healing in ACL reconstruction by MRI. No difference was observed between the BMSCs and control groups in the signal-to-noise ratio of the MRI interzone. In their study, bone marrow was harvested from the anterior iliac crest with the syringe in the MarrowStim Concentration System kit (Table 4).

BMSCs can be acquired clinically from the anterior iliac crest, posterior superior iliac crest, tibial tubercle, or sternum. A second surgery is required to obtain sufficient BMSCs, leading to further trauma and suffering. While several studies have shown promising BMSCs for the repair of human rotator cuffs, clinical trials appear to have been scarce in the recent past. These studies have some limitations, including the limited sample group, the short follow-up duration, the lack of objective measurement methods, and the lack of randomization. More randomized, large-sample, long-term trials would be needed in potential clinical studies. To determine minor changes in histology in the tendon-to-bone interface, further evaluation methods will be necessary. Furthermore, there has been no identification of the number and quality of

Table 3 Summary of pre-clinical studies using gene-modified bone marrow mesenchymal stem cells

Ref.	Animal/model	Cell/cell number	Transfected gene	Method of delivery	Control	Timeline	Outcome measurement
Gulotta *et al*[72], 2010	Rat/Unilateral detachment and repair of the supraspinatus tendon	BMSCs/× 10^6	MT1-MMP	BMSCs were pelleted down and mixed with fibrin sealant for implantation	Rotator cuff repair with normal BMSCs	2 and 4 wk	Histology, biomechanics
Gulotta *et al*[76], 2011	Rat/Unilateral detachment and repair of the supraspinatus tendon	BMSCs/× 10^6	Scx	BMSCs were pelleted down and mixed with fibrin sealant for implantation	Rotator cuff repair with normal BMSCs	2 and 4 wk	Histology, biomechanics
Gulotta *et al*[77], 2011	Rat/Unilateral detachment and repair of the supraspinatus tendon	BMSCs/× 10^6	BMP-13	BMSCs were pelleted down and mixed with fibrin sealant for implantation	Rotator cuff repair with normal BMSCs	2 and 4 wk	Histology, biomechanics
Dong *et al*[78], 2012	Rabbit/Unilateral ACL reconstructions using gastrocnemius tendon autografts	BMSCs/× 10^7	BMP-2	Grafts were coated with BMSCs in a fibrin glue carrier	ACL reconstruction without BMSCs	4 and 8 wk	Histology, biomechanics
Wang *et al*[79], 2017	Rabbit/Unilateral ACL reconstructions using flexor digitorum longus tendon autografts	BMSCs/-	TGF-β	BMSCs were injected into the bone tunnel and graft tendon cavity	ACL reconstruction with normal BMSCs	6 and 12 wk	Histology, immunohistochemistry, biomechanics, 3D-CT
Wang *et al*[80], 2018	Rat/Bilateral detachment and repair of the supraspinatus tendon	BMSCs/1.6 × 10^7	PDGF-B	BMSCs with fibrin glue implanted into the defects	Rotator cuff repair with irrelevant plasmid-transfected BMSCs	4 and 8 wk	Histology, immunohistochemistry, biomechanics

BMSC: Bone marrow mesenchymal stem cell; MT1-MMP: Membrane type 1 matrix metalloproteinase; Scx: Scleraxis; BMP-13: Bone morphogenetic protein-13; BMP-2: Bone morphogenetic protein-2; TGF-β: Transforming growth factor-beta; PDGF-B: Platelet-derived growth factor subunit B.

Table 4 Summary of clinical studies using bone marrow mesenchymal stem cells

Ref.	Study type/patient number	Cell/cell number	Method of delivery	Control	Follow up	Outcome measurement
Jo *et al*[82], 2013	Cohort study/124	BMSCs/-	Drilling into the bone marrow was performed in the greater tuberosity	Arthroscopic rotator cuff repair without drilling	At a minimum of 2 yr	VAS, ROM, muscle strength, functional scores, MRI, CTA
Hernigou *et al*[84], 2014	Case-controlled study/90	BMSCs/12 mL (51000 ± 25000 cells)	BMSCs were injected into the tendon-bone interface	Arthroscopic rotator cuff repair without BMSCs	At a minimum of 10 yr	ROM, muscle strength, MRI, ultrasound
Silva *et al*[85], 2014	RCT/43	BMSCs/-	BMSCs were injected in the tunnel	ACL reconstruction without BMSCs	3 mo	MRI
Taniguchi *et al*[83], 2015	Retrospective cohort study/111	BMSCs/-	Drilling into the bone marrow was performed in the greater tuberosity	Arthroscopic rotator cuff repair without drilling	12-24 mo	MRI

BMSC: Bone marrow mesenchymal stem cell; VAS: Visual analog scale; ROM: Range of motion; MRI: Magnetic resonance imaging; CTA: Computed tomography arthrography; RCT: Randomized controlled trials.

the recruited BMSCs and no clarity on the mechanism. In addition, tissue engineering with BMSCs has not been reported in clinical research, and further research is needed. Recently, Voss *et al*[86] designed a PRP fibrin clot, a scaffold for stem cell delivery. The technique used in the clinic was associated with a lack of clinical outcomes, however, the fibrin scaffold will retain the viability of BMSCs for lengthy periods (> 4 wk).

TSPCS

Remarkable progress has been made in the identification of human and mouse TSPCs with universal stem cell characteristics, including clonogenicity, multipotency, and self-renewal capacity[87]. Then, TSPCs have been identified in rabbits and rats[88,89]. de Mos *et al*[90] also demonstrated the multi-differentiation potential of explanted human tendon cells. Not only do these cells have universal stem cell properties similar to BMSCs, but they are also strongly characterized by tendon-connected genes and proteins, including collagen I, tenascin C, Scx, and Tnmd[87]. Although MSCs are commonly used in tendon-bone interconnection repairs isolated from various tissues, the use of tendon stem cells for the reparation of the tendon-bone interconnection may be beneficial in the light of the fact that the tendon environment is ideal and familiar to facilitate grafting and differentiation between transplanted cells[91]. More researches have recently examined the effectiveness of TSPCs in the context of tendon-bone injury.

Tissue engineering has been shown to be promising for tendon-bone junction repair by means of stem cell therapy. In the promotion of rotator cuff regeneration in a rabbit model, Shen *et al*[17] investigated the effectiveness of TSPCs seeded knitted silk-collagen sponge scaffold. The implantation of TSPCs allogenous scaffolds 4 and 8 wk post-operation increased fibroblastic cell growth and decreased lymphocyte infiltration at implantation locations. The allogenous TSPCs group exhibited improving structural and biomechanical properties at 12 wk after surgery. They showed that by differentiating into tenocytes and secreting anti-inflammatory cytokines, the allogenous TSPCs knitted silk-collagen sponge scaffold improved the efficacy of rotator cuff tendon regeneration. For cell transmission, cell sheets do not need external scaffolds. TSPCs form a cell sheet that displays greater tenogenicity and retains a high expression level of the chondroosteogenic gene by TSPCs. Lui *et al*[91] used a TSPCs sheet in a rat ACL reconstruction model. In the TSPCs group, bone volume and total volume were considerably higher after 2 and 6 wk. Semiquantitative image analysis showed improved graft osteointegration and higher intra-articular graft integrity with lower cellularity and vascularity, better cell alignment, and increased collagen birefringence in the TSPCs group. The TSPCs group displayed better results than the control group under biomechanical analysis. They concluded that after restoration of the ACL, the TSPCs sheet enhanced early graft healing. Cell sheets of human rotator cuff-derived cells were used in a rat rotator cuff injury model by Yoshifumi *et al*[92]. Proteoglycan was found to be positive for more type II collagen and isolectin-B4 cells in the sheet group, *VEGF* and type II collagen gene expression was higher, and tenomodulin gene expression was lower in the sheet group than in the control group. The sheet group displayed a considerably higher ultimate failure load in mechanical testing at 8 wk than the control group. Their results indicated that the rotator-cuff derived cell sheet could promote cartilage regeneration and angiogenesis at the enthesis, with superior mechanical strength. Although the studies support TSPCs in the repair of tendon and tendon bone insertion as an alternative cell source, no clinical research has addressed repair of following TBI.

Research concerning the mechanism by which TSPCs improve tendon-bone healing is scarce. Cheng *et al*[93] investigated the role of TNF-alpha-stimulated gene/protein 6 (TSG-6) in the treatment of rotator cuff healing with TSPCs. It was concluded that TSG-6, a therapeutic protein manufactured by TSPCs to respond to injury signals, prevented the formation of fibro-vascular tissue in the tendon-to-bone interface *via* inflammation suppression. The other mechanism is still unknown, and the actual mechanisms must be researched further.

Autologous tendon stem cells are difficult to acquire without inducing site morbidity or second stage surgery for the donor. Researchers have now shown that tendon stem cells have been immune-privileged and can be used for the transplantation of allogenes[91]. TSPCs could be easily isolated from the waste tendon tissue during tendon/ligament surgery, such as with the residual tendon graft tissue in ACL reconstruction and the waste tendon tissue in total knee replacement[91]. The study using TSPCs on tendon-bone junction injury is still in its infancy. Further research is needed to test the effectiveness of TSPCs application in a large-animal model and identify the efficacy of this *in vivo*.

SMSCS

Recently, SMSCs are becoming increasingly popular and are widely used in cell-based

tissue repair[94-96]. It has been reported that MSCs from the synovium have a higher chondrogenic potential than those from infrapatellar fat pad, adipose tissue, and bone marrow[97,98]. Therefore, SMSCs were introduced in recent years to accelerate tendon bone healing.

Ju *et al*[99] excised bilateral knee joint rat synovial membrane to provide a single-cell suspension of SMSCs and labeled it with CM-DiI. Atelocollagen gel with or without rat SMSCs was injected into the interface of bone and tendon during surgery. The interface was full of abundant DiI-labeled SMSCs at week 1, and the amount of collagen fibers in the interface had increased significantly. After 2 wk, Sharpey's fibers seemed to be slightly higher in the proportion of oblique collagen fibers between the tendon graft and bone tunnel. The tendon-bone interface tissue vanished after 4 wk, and the inserted tendon tended to connect directly to the bone. At this time, DiI-labeled cells can no longer be detected. They proposed that implanted SMSCs could differentiate into fibroblasts and accelerate early remodeling of tendon-bone healing, although they cannot regenerate a stable insertion of fibrocartilage. In conclusion, SMSCs can enhance tendon-bone healing effectively by accelerating fibrous structure formation.

Knee and shoulder joints can be used to generate SMSCs. Recently, several studies have indicated that the subacromial bursa is an effective candidate for the source of SMSCs in rotator cuff tears[98,100]. However, the use of SMSCs for clinical research is still scarce. The acquisition and cultivation of SMSCs require secondary surgery, which will burden patients financially, making it difficult to perform clinical trials. In the future, further biomechanical research for the tendon-bone complex should be conducted to assess whether the SMSC truly biomechanically improves early tendon-bone healing. This will also entail more scientific studies involving large animals. Furthermore, to investigate the therapeutic effectiveness of SMSCs, more clinical trials should be performed.

ADSCS

As a potential source of undifferentiated MSCs, adipose tissue has recently been garnering interest. The stem cell derived from adipose is a progenitor cell that can proliferate and differentiate into various types of mesenchymal cells, such as tenocytes or myocytes, and release growth factors and cytokines locally[101,102]. Since ADSCs can easily be obtained in large quantities from subcutaneous adipose tissue without the need for culture and expansion, they have been widely used in research in recent years.

Pre-clinical studies

Several animal studies have shown that the local repair and regeneration of adipose tissue, muscle, endothelial tissue, and bone can be improved by ADSCs[102-104]. In a rabbit rotator cuff tear model, Oh *et al*[105] injected ADSCs to verify the effects of tendon-bone healing. In terms of electromyographic assessment, the ADSCs group exhibited a larger compound muscle action potential area and higher load-to-failure result. They concluded that local administration of ADSCs could enhance tendon bone healing. However, only the biomechanical pullout strength was calculated with no histological assessment of tendon-bone healing. Mora *et al*[106] reported that using ADSCs in a rotator cuff repair rat model did not enhance tendon-to-bone healing biomechanical properties, but ADSCs were able to inhibit inflammatory reaction, which could result in more elastic and less scarred repair. With a diminished presence of oedema and neutrophils in the ADSCs group, histological analysis revealed less acute inflammation. But the orientation of collagen fibers and biomechanical variables in each group did not vary after 2 and 4 wk. Similar findings have been yielded by Barco *et al*[107].

Kosaka *et al*[108] evaluated the therapeutic effect of ADSCs administration on tendon-bone healing in a rabbit ACL reconstruction model. The size and type of chondroide cells and Sharpey-like fibers were more orderly and consistently; they were previously seen in the ADSC treatment group. In the ADSCs-treated group, the final failure load and stiffness were higher. They concluded that at the tendon-bone junction, local administration of ADSCs facilitated the early healing process.

Stem cell injection causes massive cell loss and a low rate of survival; the supply of MSCs in a hydrogel or scaffold will improve their productivity and help overcome these disadvantages. Lipner *et al*[109] used an aligned nanofibrous poly lactic co-glycolic acid scaffold with a gradient in mineral content; this was seeded with ADSCs

transduced with osteogenic factor BMP2 and implanted at the repair site of a rat rotator cuff model. They found that cells transmitted with BMP2 resulted in impaired healing and that this growth factor should not be used in the tendon-to-bone repair setting. Rothrauff *et al*[110] investigated the effect of ADSCs and TGF-β3 delivered in hydrogels on enthesis healing after rotator cuff tear repair in rats. They concluded that supplementing ADSCs with TGF-β3 had no added benefit. In a rat chronic rotator cuff injury model, Kaizawa *et al*[111] injected ADSCs-seeded tendon hydrogel, which is a type I collagen-rich gel derived from human tendons, at the repair site. They concluded that in a chronic rotator cuff injury model, tendon hydrogel augmentation with ADSCs does not substantially stimulate tendon bone interface healing. Shin *et al* [112] evaluated the effects of an ADSCs sheet for tendon-to-bone healing of a chronic rotator cuff tear in rats. Their laboratory research provides evidence that ADSCs are successful in repairing rotator cuff tears and that a clinically feasible stem cell delivery method for rotator cuff repair is engineered stem cells.

Although ADSCs have been effective in tendon-bone healing in many studies, the exact mechanism remains unclear. First, there are assumptions that ADSCs are capable not only of self-differentiation but also of promoting the differentiation of other endogenous stem cells by stimulating their recruitment along the appropriate lineage path[113]. Second, ADSCs can regulate biological processes such as cell proliferation, migration, differentiation, and deposition of the extracellular matrix, which can also be an important part of tendon healing through growth factors and cytokines. In addition, ADSCs can supply the isochemical site with antioxidant chemicals or free-radical scavengers to help remove toxic substances from torn tendons and musculo-skeletal tissues and to promote cell recovery.

Clinical studies

Kim *et al*[114] determined the effect of an injection of ADSCs loaded in fibrin glue during arthroscopic rotator cuff repair on clinical outcomes. Among the 182 patients, 35 underwent arthroscopic rotator cuff repair *via* the injection of ADSCs loaded fibrin glue. Outcomes were measured using the VAS for pain, ROM, and functional measurements of the Constant score and UCLA shoulder rating scale. The repaired structural integrity of the tendon was assessed using MRI. The mean clinical follow-up period was 28 mo. The findings indicated that there were no significant differences between the groups at the final follow-up of the VAS score, the internal rotation at the back, the Constant score, and UCLA score. Forward flexion and external side rotation substantially improved in the final follow-up in both groups. In the control group, MRI showed a re-tear rate of 28.5% and 14.3% in the injection group. This study showed a major improvement for structural effects with respect to the re-tear rate when injecting ADSCs loaded fibrin glue during rotator cuff repair. However, there were no clinical differences in the 28-mo follow-up period. Eduard *et al*[115] then compared the healing and clinical effects of ACL reconstruction between patients with or without intraoperative ADSCs administration. They followed 20 soccer players who underwent ACL reconstruction using a bone-patellar tendon-bone autograft infiltrated with ADSCs. Outcomes were collected at baseline and 6 and 12 mo postoperatively for the International Knee Documentation Committee (IKDC) score, Lysholm knee score, VAS, and MRI. All patients had better outcomes and returned to playing sports after surgery. They concluded that it is not justified to improve cure or accelerate the functions after the reconstruction of ACL through the intraoperative administration of ADSCs at this point.

Currently, ADSCs are still rarely used for clinical research. The number of patients was relatively small, and the duration of follow-up was limited. There was no randomized controlled trial. To confirm the effect of using ADSCs in the reconstruction of ACL and rotator cuff healing, future studies with larger sample sizes will be necessary at different times.

PERIOSTEUM-DERIVED PSCS

Numerous studies have shown that the periosteum contains multipotent stem cells called periosteum-derived PSCs to differentiate osteogenic and chondrogenic tissues [116,117]. In 1930, Burman *et al*[118] used a periosteal autograft to enhance bone formation around a tendon graft. The periosteum consists of two layers, the outer layer mainly includes fibroblasts with collagen fiber, and the inner layer contains mostly undifferentiated granular matrix precursor cells. Therefore, depending on whether the cambium side is positioned towards the bone or towards the tendon,

when a periosteum patch is transplanted into the tendon-bone interface, the outcome will vary. In the last several years, multiple researchers have investigated whether a free periosteal graft would improve tendon healing in a bone tunnel, and whether periosteal fiber and enhancements can induce progress in tendon-bone healing.

Pre-clinical studies

Karaoglu *et al*[119] compared the effect of enhancing the tendon-bone attachment between the periosteum and BMSCs and found that periosteum had a positive effect after 6 wk, whereas bone marrow was more effective after 12 wk. Youn *et al*[120] tested the different efficacy levels between periosteal graft with the fibrous layer facing the bone and the cambium layer facing the bone wrapped around the tendon. Biomechanical testing demonstrated that the fresh cambium layer facing toward the bone group had a higher ultimate failure load than any other groups. A fluorescent histological assessment showed that the fresh cambium layer facing the bone group had the most organized and significant bone formation around the bone tunnel. They concluded that a periosteal augmentation of a tendon graft could enhance the structural integrity of the tendon-bone interface, when the periosteum is placed between the tendon and bone interface with the cambium layer facing toward the bone. In an extra-articular rabbit-bone tunnel model, Chen *et al*[121] examined the effect of the periosteum-enveloping tendon on tendon-bone healing. The findings revealed a fibrous layer that developed in the periosteum between the tendon and the bone. After 4 wk, the cancellous bone lining of the bone tunnel was interdigitized with the fibrous interface tissue. Progressive new bone grew into the interface fibrous layer after 8 wk. At 12 wk, collagen fibers had become anchored between the tendon and the bone and organized with the formation of fibrocartilage. In the periosteum-enveloped group, biomechanical tests have indicated a higher maximum pullout strength at all times. Their studies suggested that the periosteum sutured on the tendon transplanted into the bone tunnel in a superior healing process and better healing strength. Using a clinically relevant rabbit model of rotator cuff tear, then they[122] investigated the effect of the periosteum on the healing of the infraspinatus tendon and bone. Histological analysis showed that during the healing process, the cambium layer of the periosteum could be a potent interface layer which is progressively mature and structured, resulting in the formation of fiber cartilage and the eventual absorption of the disrupted tendon into the bone. The biomechanical tests showed that the attachment strength had improved gradually, demonstrating the progressive integration of tendon-bone.

Recently, tissue-engineered solutions to facilitate the integration of the tendon graft into the bone tunnel have been promising. Chen *et al*[123] injected photopolymerizable hydrogel based on poly(ethylene glycol) diacrylate (PEGDA) with hyaluronic acid-tethered BMP-2 and PSCs into an extra-articular bone tunnel rabbit model. An interface fibrocartilage and new bone formed after 6 wk was examined histologically. At 3 and 6 wk after tendon transplantation, biomechanical studies revealed a greater maximum pullout strength and stiffness. Then they[124] used this injectable hydrogel made with PSCs and PEGDA tethered with BMP-2 in a rabbit model of rotator cuff tear. This study found that the tendon-bone interface had an expanded fibrocartilage and bone layer, with a maximum pull-out load at 4 and 8 wk. These studies demonstrated that PSC and PEGDA along with BMP-2, have a high inductive potential for healing between the tendon and the bone. Subsequently, they[125] developed a scaffold-free method using polymerized fibrin-coated dishes to make functional PSCs sheets. They evaluated the effect of bioengineered PSCs sheets in an extra-articular bone tunnel rabbit model. Histological staining showed that the tendon-bone junction was formed with higher fibrocartilage at 4 wk. Matured fibrocartilage and dense collagen fiber were formed at the tendon-bone interface at 8 wk. At the same time, they[126] compared the effects of a PSCs sheet and acellular collagen sheet on tendon-bone healing in a rabbit ACL reconstruction model. After 8 wk, the PSCs sheet group had a higher collagen and glycosaminoglycan deposition at the tendon bone junction, with fibrocartilage formation. In addition, in comparison with the group of cellular collagen sheets, the PSCs sheet group exhibited significantly better mechanical performance. These two studies have concluded that the PSCs sheet has a high inductive potential for tendon and bone healing.

Although numerous studies have found that PSCs can improve tendon-bone healing, they have not recognized the impact of synovial fluid and physiological loading in their animal model of the extraarticular tendon-bone healing model.

Clinical studies

As the periosteum may represent a novel treatment modality for enhancing tendon–bone healing, this technique may be used in clinical practice for ACL reconstruction or rotator cuff repair to improve tendon graft healing inside the tunnel. Chen *et al*[127] used periosteum-enveloping hamstring tendon grafts in 62 patients who underwent ACL reconstruction. They were followed for a minimal of 2 years. Clinical assessments included the Lysholm knee scores, IKDC scores, KT-1000 instrumented testing, thigh muscle assessment, and radiographic evaluation. All patients showed a higher Lysholm knee score at the end of follow-up; 92% of patients had a normal or almost normal IKDC score; 6% of patients were found to have grade 2 or more ligament laxity. Forty-six patients achieved recovery of the extensor muscle strength in the reconstructed knee to 90% or more of normal knee strength. Radiographic evaluation found that a rise of more than 1 mm was identified in three (5%) femoral tunnels and four (6%) tibial tunnels in five of 62 patients. After reconstruction, 81% of patients were able to return to moderate or strenuous activity. A few years later, this group[128] used single-bundle ACL reconstruction with periosteum-enveloping hamstring tendon grafts on 368 patients. Those patients were followed for an average of 4.6 years. There have been similar findings which led to the conclusion that the periosteum-enveloping hamstring tendon graft in ACL rebuilding would achieve a satisfactory outcome.

The clinical and radiological outcomes of open rotator cuff reconstruction were then examined by Scheibel *et al*[129] in 23 patients with a mean follow-up period of 14.4 mo, using an autologous periosteal flap augmentation technique. The Constant-Score rose dramatically and a significant positive result was yielded by the Simple Shoulder test. In postoperative MRI, only four (20%) patients displayed a re-tear of the tendon. They concluded that the autologous periosteal flap in the open rotator cuff repair has a high patient level of satisfaction with low re-rupture rates.

Although these studies have achieved satisfactory results, they were performed as prospective studies with fewer samples and a shorter follow-up time. In the future, it will be necessary to compare and further evaluate the potential benefits of periosteal improvements in ACL or rotator cuff healing in clinical prospective randomized trials with large samples.

STEM CELLS DERIVED FROM ACL TISSUES

The existence of MSC–like cells in human ACL tissues has been discovered recently. Matsumoto *et al*[130] first isolated vascular stem cells with characteristic expression of CD34 in the injured ACL tissues in 2012. They demonstrated that ACL ruptured tissue contained abundant CD34-positive cells (44.3%) compared with intact ACL tissue (8.3%), and CD34-positive cells exhibited multilineage differentiation capacities, including the ability to undergo chondrogenesis, osteogenesis, adipogenesis, and endotheliogenesis. The canine ACL reconstruction model was then used to demonstrate that ACL injured tissue has the therapeutic potential to foster a suitable environment for tendon-to-bone healing in bone tunnels of ACL reconstruction[131].

Mifune *et al*[132] injected CD34+ stem cells derived from adult human ACL into the articular cavity in nude rats after ACL reconstruction in the same year. A greater area of collagen fiber formation and more collagen type II expression in the CD34+ group were revealed by a histological evaluation. The immunostaining study showed that CD34+ stem cells derived from ACL had a greater capacity to differentiate into endothelial cells and osteoblasts. After the treatment of CD34+ cells, the biomechanical test showed the failure load to exhibit almost the same strength as the uninjured ACL. Subsequently, they advanced the study by using a cell sheet wrapped graft with ACL-derived CD34+ cells in a nude rat ACL reconstruction model, which also exhibited promising healing potential[133].

Although some studies have already proven that ACL-derived CD34-positive cells contribute to tendon-bone healing after ACL reconstruction, the relationship between the healing potential of ACL-derived cells and the patient's age or elapsed time after injury remains uncertain. Nakano *et al*[134] injected ACL-derived CD34+ stem cells from patients aged 10 to 19 years (younger group) or patients aged 30 to 39 years (older group) into the ACL reconstruction model of immunodeficient rats. A significantly higher ultimate load to failure rate and a greater ability of differentiation into endothelial cells and osteoblasts were observed in the younger group. They also demonstrated that in the immunodeficient rat model of ACL reconstruction, ACL-derived cells from young patients would have a greater effect on the maturation of

tendon-bone integration compared to cells from older patients. A few years later, Inokuchi *et al*[135] harvested ACL-derived CD34+ stem cells from two groups of patients, namely, an early group (≤ 3 mo after injury) and a chronic group (≥ 3 mo after injury), based on the time that elapsed between injury and surgery. They then tested the influence of elapsed time after injury on the healing process in an immunodeficient rat model of ACL reconstruction; they concluded that cells obtained during the early phase after injury have a higher tendon-bone healing potential compared to human ACL-derived cells obtained during the chronic phase.

Recent studies have conducted the genetic modification of stem cells derived from ACL. A cell sheet-wrapped graft with ACL-derived CD34+ cells expressing either VEGF or soluble FLT-1 was used by Takayama *et al*[136] in the ACL reconstruction model of nude rats. It was concluded that the indigenous expression of VEGF in the CD34+ cells improved tendon graft maturation and biomechanical strength, but that the overexpression of VEGF prevented improvements in biomechanical strength. Kawakami *et al*[137] used adult human ACL-derived CD34+ cells transduced with BMP-2 in a rat model, and demonstrated that ACL-derived CD34+ cells transduced with BMP-2 accelerated graft-bone integration after ACL reconstruction, as evidenced by the enhanced histological appearance and graft-bone interface biology along with tensile load to failure.

Although current research has shown that ACL-derived CD34+ cells contributed to the tendon-bone healing after ACL reconstruction by enhancing angiogenesis and osteogenesis after ACL reconstruction, which also enhances biomechanical strength [132,133], clinical trials are not yet available, and some limitations remain. First, rats have a higher healing ability than humans, so the grafting progress of tendon healing in rats is likely to advance faster than in humans. Second, current studies have not indicated whether stem cells derived from ACL could differentiate into fibroblasts to directly contribute to the graft tendon healing[132]. Second-step arthroscopic surgery is inevitable due to the need for cell isolation, cell culture, and cell expansion when cell therapy is applied for ACL reconstruction, thereby affecting the clinical viability of CD34+ cell transplantation[131]. More experiments are necessary to confirm its clinical viability and the actual mechanisms of this application in a larger animal model must be further investigated.

LIMITATIONS OF STEM CELL THERAPY AND DIRECTIONS FOR FUTURE RESEARCH

Despite the promising consequences of stem cell therapy, there are quite a few limitations. The most widely used methods to move stem cells into the bone tunnel are direct intra-articular injection; thus cell leakage is inevitable. In tendon and bone healing implant MSCs, the biological process is unclear and needs to be further clarified. For gene therapy, one of the key drawbacks is the decline in gene expression after several weeks, which could result in the loss of the desired product. Moreover, there is no guarantee regarding the safety of gene therapy, as mutagenesis, malignancy, and other side-effects may occur. Based on animal research, the short-term impact of stem cell therapy has been reasonably successful. However, there is still no long-term impact or confirmation based on human studies.

Regarding the clinical applications, the dosage and duration of stem cell therapy have not been achieved to ensure the best result. A two-stage arthroscopic surgery is inevitable for a sufficient number of autologous stem cells, which will result in unnecessary pain and costs. It is important to develop a more effective and cheaper surgical strategy. Most stem cell therapy trials are focused on small animal models, which have a higher rate of tendon-bone healing than in human patients. To prove its clinical viability, further studies to test the efficacy of this application in large animal models are needed. In addition to the effectiveness of repair and regeneration of stem cells, it is important to properly assess possible hazards or adverse events, such as carcinogenesis, prior to clinical use.

CONCLUSION

MSCs may be particularly useful for stem cell-based therapies for several reasons. First, MSCs of different mesenchymal tissues have been insulated, including bone marrow, muscle, circulatory blood, blood vessels, and fat[138]. Second, MSCs can be

differentiated into various cell types, such as osteoblasts, chondrocytes, and adipocytes [139]. This indicates that relative to other adult stem cells, MSCs could have broader therapeutic applications. Third, implanted BMSCs extend into ligament fibroblast and cell fibrocartilage, which leads to fibrocartilage accumulation. Forth, MSCs exert strong paracrine effects, boosting the ability of tissue damage to self-repair.

Moreover, the fibrocartilage interface is also derivative of local cells, and injected MSCs can secrete various growth factors to promote local fibroblast and fibro-cartilage cell activation and recruitment.

In conclusion, the acceleration of tendon-bone healing has proven to be successful for many types of stem cells. MSCs, due to their high rate of proliferation, multipower, and relative ease of separation from diverse tissues, are highly appealing for the promotion of tendon-bone junction healing. However, these findings were yielded under restricted conditions and clinical evidence. In future trials, numerous issues, including application requirements and technological and safety problems, must be addressed prior to clinical application. Meanwhile, research on the biological healing mechanism of the stem cells implanted for tendon-to-bone healing is also required.

REFERENCES

1   **Maffulli N**, Wong J, Almekinders LC. Types and epidemiology of tendinopathy. *Clin Sports Med* 2003; **22**: 675-692 [PMID: 14560540 DOI: 10.1016/s0278-5919(03)00004-8]

2   **Galatz LM**, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am* 2004; **86**: 219-224 [PMID: 14960664 DOI: 10.2106/00004623-200402000-00002]

3   **Vergis A**, Gillquist J. Graft failure in intra-articular anterior cruciate ligament reconstructions: a review of the literature. *Arthroscopy* 1995; **11**: 312-321 [PMID: 7632308 DOI: 10.1016/0749-8063(95)90009-8]

4   **Harryman DT 2nd**, Mack LA, Wang KY, Jackins SE, Richardson ML, Matsen FA 3rd. Repairs of the rotator cuff. Correlation of functional results with integrity of the cuff. *J Bone Joint Surg Am* 1991; **73**: 982-989 [PMID: 1874784]

5   **Musahl V**, Karlsson J. Anterior Cruciate Ligament Tear. *N Engl J Med* 2019; **380**: 2341-2348 [PMID: 31189037 DOI: 10.1056/NEJMcp1805931]

6   **Levine JW**, Kiapour AM, Quatman CE, Wordeman SC, Goel VK, Hewett TE, Demetropoulos CK. Clinically relevant injury patterns after an anterior cruciate ligament injury provide insight into injury mechanisms. *Am J Sports Med* 2013; **41**: 385-395 [PMID: 23144366 DOI: 10.1177/0363546512465167]

7   **Grana WA**, Egle DM, Mahnken R, Goodhart CW. An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. *Am J Sports Med* 1994; **22**: 344-351 [PMID: 8037275 DOI: 10.1177/036354659402200309]

8   **Rodeo SA**, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF. Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. *J Bone Joint Surg Am* 1993; **75**: 1795-1803 [PMID: 8258550 DOI: 10.1007/978-1-4471-5451-8_115]

9   **Rougraff B**, Shelbourne KD, Gerth PK, Warner J. Arthroscopic and histologic analysis of human patellar tendon autografts used for anterior cruciate ligament reconstruction. *Am J Sports Med* 1993; **21**: 277-284 [PMID: 8465925 DOI: 10.1177/036354659302100219]

10   **Rothrauff BB**, Tuan RS. Cellular therapy in bone-tendon interface regeneration. *Organogenesis* 2014; **10**: 13-28 [PMID: 24326955 DOI: 10.4161/org.27404]

11   **Butler DL**, Juncosa N, Dressler MR. Functional efficacy of tendon repair processes. *Annu Rev Biomed Eng* 2004; **6**: 303-329 [PMID: 15255772 DOI: 10.1146/annurev.bioeng.6.040803.140240]

12   **Wong MW**, Qin L, Tai JK, Lee SK, Leung KS, Chan KM. Engineered allogeneic chondrocyte pellet for reconstruction of fibrocartilage zone at bone-tendon junction--a preliminary histological observation. *J Biomed Mater Res B Appl Biomater* 2004; **70**: 362-367 [PMID: 15264320 DOI: 10.1002/jbm.b.30049]

13   **Kohno T**, Ishibashi Y, Tsuda E, Kusumi T, Tanaka M, Toh S. Immunohistochemical demonstration of growth factors at the tendon-bone interface in anterior cruciate ligament reconstruction using a rabbit model. *J Orthop Sci* 2007; **12**: 67-73 [PMID: 17260120 DOI: 10.1007/s00776-006-1088-8]

14   **Anderson K**, Seneviratne AM, Izawa K, Atkinson BL, Potter HG, Rodeo SA. Augmentation of tendon healing in an intraarticular bone tunnel with use of a bone growth factor. *Am J Sports Med* 2001; **29**: 689-698 [PMID: 11734478 DOI: 10.1177/03635465010290060301]

15   **Spindler KP**, Murray MM, Carey JL, Zurakowski D, Fleming BC. The use of platelets to affect functional healing of an anterior cruciate ligament (ACL) autograft in a caprine ACL reconstruction model. *J Orthop Res* 2009; **27**: 631-638 [PMID: 19009602 DOI: 10.1002/jor.20785]

16   **Kida Y**, Morihara T, Matsuda K, Kajikawa Y, Tachiiri H, Iwata Y, Sawamura K, Yoshida A, Oshima Y, Ikeda T, Fujiwara H, Kawata M, Kubo T. Bone marrow-derived cells from the footprint infiltrate into the repaired rotator cuff. *J Shoulder Elbow Surg* 2013; **22**: 197-205 [PMID: 22543003 DOI: 10.1016/j.jse.2012.02.007]

17   **Shen W**, Chen J, Yin Z, Chen X, Liu H, Heng BC, Chen W, Ouyang HW. Allogenous tendon stem/progenitor cells in silk scaffold for functional shoulder repair. *Cell Transplant* 2012; **21**: 943-958 [PMID: 22405331 DOI: 10.3727/096368911X627453]

18   **Zhu Z**, Yu A, Hou M, Xie X, Li P. Effects of Sox9 gene therapy on the healing of bone-tendon junction: An experimental study. *Indian J Orthop* 2014; **48**: 88-95 [PMID: 24600069 DOI: 10.4103/0019-5413.125521]

19   **Petrigliano FA**, McAllister DR, Wu BM. Tissue engineering for anterior cruciate ligament reconstruction: a review of current strategies. *Arthroscopy* 2006; **22**: 441-451 [PMID: 16581458 DOI: 10.1016/j.arthro.2006.01.017]

20   **Atesok K**, Fu FH, Wolf MR, Ochi M, Jazrawi LM, Doral MN, Lubowitz JH, Rodeo SA. Augmentation of tendon-to-bone healing. *J Bone Joint Surg Am* 2014; **96**: 513-521 [PMID: 24647509 DOI: 10.2106/JBJS.M.00009]

21   **Genin GM**, Thomopoulos S. The tendon-to-bone attachment: Unification through disarray. *Nat Mater* 2017; **16**: 607-608 [PMID: 28541313 DOI: 10.1038/nmat4906]

22   **Benjamin M**, Toumi H, Ralphs JR, Bydder G, Best TM, Milz S. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. *J Anat* 2006; **208**: 471-490 [PMID: 16637873 DOI: 10.1111/j.1469-7580.2006.00540.x]

23   **Benjamin M**, Evans EJ, Copp L. The histology of tendon attachments to bone in man. *J Anat* 1986; **149**: 89-100 [PMID: 3693113]

24   **Benjamin M**, Ralphs JR. Tendons and ligaments--an overview. *Histol Histopathol* 1997; **12**: 1135-1144 [PMID: 9302572]

25   **Buehler MJ**, Yung YC. Deformation and failure of protein materials in physiologically extreme conditions and disease. *Nat Mater* 2009; **8**: 175-188 [PMID: 19229265 DOI: 10.1038/nmat2387]

26   **Waggett AD**, Ralphs JR, Kwan AP, Woodnutt D, Benjamin M. Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. *Matrix Biol* 1998; **16**: 457-470 [PMID: 9550263 DOI: 10.1016/s0945-053x(98)90017-8]

27   **Wang IE**, Mitroo S, Chen FH, Lu HH, Doty SB. Age-dependent changes in matrix composition and organization at the ligament-to-bone insertion. *J Orthop Res* 2006; **24**: 1745-1755 [PMID: 16779829 DOI: 10.1002/jor.20149]

28   **Benjamin M**, Kumai T, Milz S, Boszczyk BM, Boszczyk AA, Ralphs JR. The skeletal attachment of tendons--tendon "entheses". *Comp Biochem Physiol A Mol Integr Physiol* 2002; **133**: 931-945 [PMID: 12485684 DOI: 10.1016/s1095-6433(02)00138-1]

29   **Liu Y**, Thomopoulos S, Chen C, Birman V, Buehler MJ, Genin GM. Modelling the mechanics of partially mineralized collagen fibrils, fibres and tissue. *J R Soc Interface* 2014; **11**: 20130835 [PMID: 24352669 DOI: 10.1098/rsif.2013.0835]

30   **Schiavone Panni A**, Fabbriciani C, Delcogliano A, Franzese S. Bone-ligament interaction in patellar tendon reconstruction of the ACL. *Knee Surg Sports Traumatol Arthrosc* 1993; **1**: 4-8 [PMID: 8535996 DOI: 10.1007/BF01552150]

31   **Liu SH**, Panossian V, al-Shaikh R, Tomin E, Shepherd E, Finerman GA, Lane JM. Morphology and matrix composition during early tendon to bone healing. *Clin Orthop Relat Res* 1997; 253-260 [PMID: 9186227 DOI: 10.1097/00003086-199706000-00034]

32   **Robert H**, Es-Sayeh J, Heymann D, Passuti N, Eloit S, Vaneenoge E. Hamstring insertion site healing after anterior cruciate ligament reconstruction in patients with symptomatic hardware or repeat rupture: a histologic study in 12 patients. *Arthroscopy* 2003; **19**: 948-954 [PMID: 14608313 DOI: 10.1016/j.arthro.2003.09.007]

33   **Ishibashi Y**, Toh S, Okamura Y, Sasaki T, Kusumi T. Graft incorporation within the tibial bone tunnel after anterior cruciate ligament reconstruction with bone-patellar tendon-bone autograft. *Am J Sports Med* 2001; **29**: 473-479 [PMID: 11476389 DOI: 10.1177/03635465010290041601]

34   **Pinczewski LA**, Clingeleffer AJ, Otto DD, Bonar SF, Corry IS. Integration of hamstring tendon graft with bone in reconstruction of the anterior cruciate ligament. *Arthroscopy* 1997; **13**: 641-643 [PMID: 9343656 DOI: 10.1016/s0749-8063(97)90194-8]

35   **Lui P**, Zhang P, Chan K, Qin L. Biology and augmentation of tendon-bone insertion repair. *J Orthop Surg Res* 2010; **5**: 59 [PMID: 20727196 DOI: 10.1186/1749-799X-5-59]

36   **Haus J**, Refior HJ. A study of the synovial and ligamentous structure of the anterior cruciate ligament. *Int Orthop* 1987; **11**: 117-124 [PMID: 3610404 DOI: 10.1007/BF00266696]

37   **Deehan DJ**, Cawston TE. The biology of integration of the anterior cruciate ligament. *J Bone Joint Surg Br* 2005; **87**: 889-895 [PMID: 15972898 DOI: 10.1302/0301-620X.87B7.16038]

38   **Carofino B**, Fulkerson J. Medial hamstring tendon regeneration following harvest for anterior cruciate ligament reconstruction: fact, myth, and clinical implication. *Arthroscopy* 2005; **21**: 1257-1265 [PMID: 16226656 DOI: 10.1016/j.arthro.2005.07.002]

39   **Fan J**, Varshney RR, Ren L, Cai D, Wang DA. Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration. *Tissue Eng Part B Rev* 2009; **15**: 75-86 [PMID: 19196118 DOI: 10.1089/ten.teb.2008.0586]

40   **Yang LF**, Liu W, Zhou Y, Feng ZS, Zhang L, Zhao W, Li Z. Human placenta-derived mesenchymal stem cell transplantation promotes tendon graft healing in a bone tunnel. *Zhongguo Zuzhi Gongcheng Yanjiu* 2013; **17**: 8539-8544 [DOI: 10.3969/j.issn.2095-4344.2013.49.012]

41   **De Bari C**, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 2001; **44**: 1928-1942 [PMID: 11508446 DOI:

10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P]

42   **De Bari C**, Dell'Accio F, Luyten FP. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis Rheum* 2001; **44**: 85-95 [PMID: 11212180 DOI: 10.1002/1529-0131(200101)44:1<85::AID-ANR12>3.0.CO;2-6]

43   **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295 [PMID: 12475952 DOI: 10.1091/mbc.e02-02-0105]

44   **Banfi A**, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R. Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. *Exp Hematol* 2000; **28**: 707-715 [PMID: 10880757 DOI: 10.1016/s0301-472x(00)00160-0]

45   **Tan Q**, Lui PP, Rui YF, Wong YM. Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering. *Tissue Eng Part A* 2012; **18**: 840-851 [PMID: 22011320 DOI: 10.1089/ten.TEA.2011.0362]

46   **Sakaguchi Y**, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum* 2005; **52**: 2521-2529 [PMID: 16052568 DOI: 10.1002/art.21212]

47   **Shirasawa S**, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells. *J Cell Biochem* 2006; **97**: 84-97 [PMID: 16088956 DOI: 10.1002/jcb.20546]

48   **Yoshimura H**, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res* 2007; **327**: 449-462 [PMID: 17053900 DOI: 10.1007/s00441-006-0308-z]

49   **Rodeo SA**, Suzuki K, Deng XH, Wozney J, Warren RF. Use of recombinant human bone morphogenetic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med* 1999; **27**: 476-488 [PMID: 10424218 DOI: 10.1177/03635465990270041201]

50   **Jorgensen C**, Djouad F, Apparailly F, Noël D. Engineering mesenchymal stem cells for immunotherapy. *Gene Ther* 2003; **10**: 928-931 [PMID: 12732877 DOI: 10.1038/sj.gt.3302019]

51   **Tuan RS**, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. *Arthritis Res Ther* 2003; **5**: 32-45 [PMID: 12716446 DOI: 10.1186/ar614]

52   **Lim JK**, Hui J, Li L, Thambyah A, Goh J, Lee EH. Enhancement of tendon graft osteointegration using mesenchymal stem cells in a rabbit model of anterior cruciate ligament reconstruction. *Arthroscopy* 2004; **20**: 899-910 [PMID: 15525922 DOI: 10.1016/j.arthro.2004.06.035]

53   **Ouyang HW**, Goh JC, Lee EH. Use of bone marrow stromal cells for tendon graft-to-bone healing: histological and immunohistochemical studies in a rabbit model. *Am J Sports Med* 2004; **32**: 321-327 [PMID: 14977654 DOI: 10.1177/0095399703258682]

54   **Soon MY**, Hassan A, Hui JH, Goh JC, Lee EH. An analysis of soft tissue allograft anterior cruciate ligament reconstruction in a rabbit model: a short-term study of the use of mesenchymal stem cells to enhance tendon osteointegration. *Am J Sports Med* 2007; **35**: 962-971 [PMID: 17400750 DOI: 10.1177/0363546507300057]

55   **Hur CI**, Ahn HW, Seon JK, Song EK, Kim GE. Mesenchymal Stem Cells Decrease Tunnel Widening of Anterior Cruciate Ligament Reconstruction in Rabbit Model. *Int J Stem Cells* 2019; **12**: 162-169 [PMID: 30595005 DOI: 10.15283/ijsc18022]

56   **Li YG**, Wei JN, Lu J, Wu XT, Teng GJ. Labeling and tracing of bone marrow mesenchymal stem cells for tendon-to-bone tunnel healing. *Knee Surg Sports Traumatol Arthrosc* 2011; **19**: 2153-2158 [PMID: 21503809 DOI: 10.1007/s00167-011-1506-0]

57   **Nourissat G**, Diop A, Maurel N, Salvat C, Dumont S, Pigenet A, Gosset M, Houard X, Berenbaum F. Mesenchymal stem cell therapy regenerates the native bone-tendon junction after surgical repair in a degenerative rat model. *PLoS One* 2010; **5**: e12248 [PMID: 20805884 DOI: 10.1371/journal.pone.0012248]

58   **Degen RM**, Carbone A, Carballo C, Zong J, Chen T, Lebaschi A, Ying L, Deng XH, Rodeo SA. The Effect of Purified Human Bone Marrow-Derived Mesenchymal Stem Cells on Rotator Cuff Tendon Healing in an Athymic Rat. *Arthroscopy* 2016; **32**: 2435-2443 [PMID: 27282111 DOI: 10.1016/j.arthro.2016.04.019]

59   **Lu J**, Chamberlain CS, Ji ML, Saether EE, Leiferman EM, Li WJ, Vanderby R. Tendon-to-Bone Healing in a Rat Extra-articular Bone Tunnel Model: A Comparison of Fresh Autologous Bone Marrow and Bone Marrow-Derived Mesenchymal Stem Cells. *Am J Sports Med* 2019; **47**: 2729-2736 [PMID: 31339739 DOI: 10.1177/0363546519862284]

60   **Gulotta LV**, Kovacevic D, Ehteshami JR, Dagher E, Packer JD, Rodeo SA. Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair model. *Am J Sports Med* 2009; **37**: 2126-2133 [PMID: 19684297 DOI: 10.1177/0363546509339582]

61   **Kanazawa T**, Soejima T, Noguchi K, Tabuchi K, Noyama M, Nakamura K, Shiba N. Tendon-to-bone healing using autologous bone marrow-derived mesenchymal stem cells in ACL reconstruction without a tibial bone tunnel-A histological study-. *Muscles Ligaments Tendons J* 2014; **4**: 201-206 [PMID: 25332936]

62   **Teng C**, Zhou C, Xu D, Bi F. Combination of platelet-rich plasma and bone marrow mesenchymal stem cells enhances tendon-bone healing in a rabbit model of anterior cruciate ligament reconstruction. *J Orthop Surg Res* 2016; **11**: 96 [PMID: 27605093 DOI: 10.1186/s13018-016-0433-7]

63   **Han L**, Fang WL, Jin B, Xu SC, Zheng X, Hu YG. Enhancement of tendon-bone healing after rotator cuff injuries using combined therapy with mesenchymal stem cells and platelet rich plasma. *Eur Rev Med Pharmacol Sci* 2019; **23**: 9075-9084 [PMID: 31696498 DOI: 10.26355/eurrev_201910_19310]

64   **Setiawati R**, Utomo DN, Rantam FA, Ifran NN, Budhiparama NC. Early Graft Tunnel Healing After Anterior Cruciate Ligament Reconstruction With Intratunnel Injection of Bone Marrow Mesenchymal Stem Cells and Vascular Endothelial Growth Factor. *Orthop J Sports Med* 2017; **5**: 2325967117708548 [PMID: 28680888 DOI: 10.1177/2325967117708548]

65   **Thangarajah T**, Sanghani-Kerai A, Henshaw F, Lambert SM, Pendegrass CJ, Blunn GW. Application of a Demineralized Cortical Bone Matrix and Bone Marrow-Derived Mesenchymal Stem Cells in a Model of Chronic Rotator Cuff Degeneration. *Am J Sports Med* 2018; **46**: 98-108 [PMID: 28949253 DOI: 10.1177/0363546517727512]

66   **Pietrzak WS**, Dow M, Gomez J, Soulvie M, Tsiagalis G. The in vitro elution of BMP-7 from demineralized bone matrix. *Cell Tissue Bank* 2012; **13**: 653-661 [PMID: 22200971 DOI: 10.1007/s10561-011-9286-9]

67   **Liu Q**, Yu Y, Reisdorf RL, Qi J, Lu CK, Berglund LJ, Amadio PC, Moran SL, Steinmann SP, An KN, Gingery A, Zhao C. Engineered tendon-fibrocartilage-bone composite and bone marrow-derived mesenchymal stem cell sheet augmentation promotes rotator cuff healing in a non-weight-bearing canine model. *Biomaterials* 2019; **192**: 189-198 [PMID: 30453215 DOI: 10.1016/j.biomaterials.2018.10.037]

68   **Omae H**, Zhao C, Sun YL, An KN, Amadio PC. Multilayer tendon slices seeded with bone marrow stromal cells: a novel composite for tendon engineering. *J Orthop Res* 2009; **27**: 937-942 [PMID: 19105224 DOI: 10.1002/jor.20823]

69   **EL Andaloussi S**, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov* 2013; **12**: 347-357 [PMID: 23584393 DOI: 10.1038/nrd3978]

70   **Shi Y**, Kang X, Wang Y, Bian X, He G, Zhou M, Tang K. Exosomes Derived from Bone Marrow Stromal Cells (BMSCs) Enhance Tendon-Bone Healing by Regulating Macrophage Polarization. *Med Sci Monit* 2020; **26**: e923328 [PMID: 32369458 DOI: 10.12659/MSM.923328]

71   **Huang Y**, He B, Wang L, Yuan B, Shu H, Zhang F, Sun L. Bone marrow mesenchymal stem cell-derived exosomes promote rotator cuff tendon-bone healing by promoting angiogenesis and regulating M1 macrophages in rats. *Stem Cell Res Ther* 2020; **11**: 496 [PMID: 33239091 DOI: 10.1186/s13287-020-02005-x]

72   **Gulotta LV**, Kovacevic D, Montgomery S, Ehteshami JR, Packer JD, Rodeo SA. Stem cells genetically modified with the developmental gene MT1-MMP improve regeneration of the supraspinatus tendon-to-bone insertion site. *Am J Sports Med* 2010; **38**: 1429-1437 [PMID: 20400753 DOI: 10.1177/0363546510361235]

73   **Holmbeck K**, Bianco P, Chrysovergis K, Yamada S, Birkedal-Hansen H. MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: a critical process in skeletal growth. *J Cell Biol* 2003; **163**: 661-671 [PMID: 14610065 DOI: 10.1083/jcb.200307061]

74   **Apte SS**, Fukai N, Beier DR, Olsen BR. The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. *J Biol Chem* 1997; **272**: 25511-25517 [PMID: 9325265 DOI: 10.1074/jbc.272.41.25511]

75   **Asou Y**, Nifuji A, Tsuji K, Shinomiya K, Olson EN, Koopman P, Noda M. Coordinated expression of scleraxis and Sox9 genes during embryonic development of tendons and cartilage. *J Orthop Res* 2002; **20**: 827-833 [PMID: 12168674 DOI: 10.1016/S0736-0266(01)00169-3]

76   **Gulotta LV**, Kovacevic D, Packer JD, Deng XH, Rodeo SA. Bone marrow-derived mesenchymal stem cells transduced with scleraxis improve rotator cuff healing in a rat model. *Am J Sports Med* 2011; **39**: 1282-1289 [PMID: 21335341 DOI: 10.1177/0363546510395485]

77   **Gulotta LV**, Kovacevic D, Packer JD, Ehteshami JR, Rodeo SA. Adenoviral-mediated gene transfer of human bone morphogenetic protein-13 does not improve rotator cuff healing in a rat model. *Am J Sports Med* 2011; **39**: 180-187 [PMID: 20956264 DOI: 10.1177/0363546510379339]

78   **Dong Y**, Zhang Q, Li Y, Jiang J, Chen S. Enhancement of tendon-bone healing for anterior cruciate ligament (ACL) reconstruction using bone marrow-derived mesenchymal stem cells infected with BMP-2. *Int J Mol Sci* 2012; **13**: 13605-13620 [PMID: 23202970 DOI: 10.3390/ijms131013605]

79   **Wang R**, Xu B, Xu HG. Up-Regulation of TGF-β Promotes Tendon-to-Bone Healing after Anterior Cruciate Ligament Reconstruction using Bone Marrow-Derived Mesenchymal Stem Cells through the TGF-β/MAPK Signaling Pathway in a New Zealand White Rabbit Model. *Cell Physiol Biochem* 2017; **41**: 213-226 [PMID: 28214835 DOI: 10.1159/000456046]

80   **Wang LL**, Yin XF, Chu XC, Zhang YB, Gong XN. Platelet-derived growth factor subunit B is required for tendon-bone healing using bone marrow-derived mesenchymal stem cells after rotator cuff repair in rats. *J Cell Biochem* 2018; **119**: 8897-8908 [PMID: 30105826 DOI: 10.1002/jcb.27143]

81   **Jo CH**, Yoon KS, Lee JH, Kang SB, Han HS, Rhee SH, Shin JS. The effect of multiple channeling on the structural integrity of repaired rotator cuff. *Knee Surg Sports Traumatol Arthrosc* 2011; **19**: 2098-2107 [PMID: 21533535 DOI: 10.1007/s00167-011-1520-2]

82   **Jo CH**, Shin JS, Park IW, Kim H, Lee SY. Multiple channeling improves the structural integrity of rotator cuff repair. *Am J Sports Med* 2013; **41**: 2650-2657 [PMID: 23942284 DOI:

10.1177/0363546513499138]

83   **Taniguchi N**, Suenaga N, Oizumi N, Miyoshi N, Yamaguchi H, Inoue K, Chosa E. Bone marrow stimulation at the footprint of arthroscopic surface-holding repair advances cuff repair integrity. *J Shoulder Elbow Surg* 2015; **24**: 860-866 [PMID: 25487905 DOI: 10.1016/j.jse.2014.09.031]

84   **Hernigou P**, Flouzat Lachaniette CH, Delambre J, Zilber S, Duffiet P, Chevallier N, Rouard H. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. *Int Orthop* 2014; **38**: 1811-1818 [PMID: 24913770 DOI: 10.1007/s00264-014-2391-1]

85   **Silva A**, Sampaio R, Fernandes R, Pinto E. Is there a role for adult non-cultivated bone marrow stem cells in ACL reconstruction? *Knee Surg Sports Traumatol Arthrosc* 2014; **22**: 66-71 [PMID: 23117168 DOI: 10.1007/s00167-012-2279-9]

86   **Voss A**, McCarthy MB, Allen D, Cote MP, Beitzel K, Imhoff AB, Mazzocca AD. Fibrin Scaffold as a Carrier for Mesenchymal Stem Cells and Growth Factors in Shoulder Rotator Cuff Repair. *Arthrosc Tech* 2016; **5**: e447-e451 [PMID: 27656360 DOI: 10.1016/j.eats.2016.01.029]

87   **Bi Y**, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med* 2007; **13**: 1219-1227 [PMID: 17828274 DOI: 10.1038/nm1630]

88   **Zhang J**, Wang JH. Characterization of differential properties of rabbit tendon stem cells and tenocytes. *BMC Musculoskelet Disord* 2010; **11**: 10 [PMID: 20082706 DOI: 10.1186/1471-2474-11-10]

89   **Rui YF**, Lui PP, Li G, Fu SC, Lee YW, Chan KM. Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng Part A* 2010; **16**: 1549-1558 [PMID: 20001227 DOI: 10.1089/ten.TEA.2009.0529]

90   **de Mos M**, Koevoet WJ, Jahr H, Verstegen MM, Heijboer MP, Kops N, van Leeuwen JP, Weinans H, Verhaar JA, van Osch GJ. Intrinsic differentiation potential of adolescent human tendon tissue: an in-vitro cell differentiation study. *BMC Musculoskelet Disord* 2007; **8**: 16 [PMID: 17319938 DOI: 10.1186/1471-2474-8-16]

91   **Lui PP**, Wong OT. Tendon stem cells: experimental and clinical perspectives in tendon and tendon-bone junction repair. *Muscles Ligaments Tendons J* 2012; **2**: 163-168 [PMID: 23738293]

92   **Harada Y**, Mifune Y, Inui A, Sakata R, Muto T, Takase F, Ueda Y, Kataoka T, Kokubu T, Kuroda R, Kurosaka M. Rotator cuff repair using cell sheets derived from human rotator cuff in a rat model. *J Orthop Res* 2017; **35**: 289-296 [PMID: 27171575 DOI: 10.1002/jor.23289]

93   **Cheng B**, Ge H, Zhou J, Zhang Q. TSG-6 mediates the effect of tendon derived stem cells for rotator cuff healing. *Eur Rev Med Pharmacol Sci* 2014; **18**: 247-251 [PMID: 24488915]

94   **De Bari C**, Dell'Accio F, Vandenabeele F, Vermeesch JR, Raymackers JM, Luyten FP. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J Cell Biol* 2003; **160**: 909-918 [PMID: 12629053 DOI: 10.1083/jcb.200212064]

95   **Ozeki N**, Muneta T, Matsuta S, Koga H, Nakagawa Y, Mizuno M, Tsuji K, Mabuchi Y, Akazawa C, Kobayashi E, Saito T, Sekiya I. Synovial mesenchymal stem cells promote meniscus regeneration augmented by an autologous Achilles tendon graft in a rat partial meniscus defect model. *Stem Cells* 2015; **33**: 1927-1938 [PMID: 25993981 DOI: 10.1002/stem.2030]

96   **Nakagawa Y**, Muneta T, Kondo S, Mizuno M, Takakuda K, Ichinose S, Tabuchi T, Koga H, Tsuji K, Sekiya I. Synovial mesenchymal stem cells promote healing after meniscal repair in microminipigs. *Osteoarthritis Cartilage* 2015; **23**: 1007-1017 [PMID: 25683149 DOI: 10.1016/j.joca.2015.02.008]

97   **Sasaki A**, Mizuno M, Ozeki N, Katano H, Otabe K, Tsuji K, Koga H, Mochizuki M, Sekiya I. Canine mesenchymal stem cells from synovium have a higher chondrogenic potential than those from infrapatellar fat pad, adipose tissue, and bone marrow. *PLoS One* 2018; **13**: e0202922 [PMID: 30138399 DOI: 10.1371/journal.pone.0202922]

98   **Utsunomiya H**, Sekiya I, Uchida S. Editorial Commentary: Are We Ready to Apply Stem Cell Therapy in Rotator Cuff Tear Surgery? *Arthroscopy* 2020; **36**: 86-87 [PMID: 31864604 DOI: 10.1016/j.arthro.2019.09.001]

99   **Ju YJ**, Muneta T, Yoshimura H, Koga H, Sekiya I. Synovial mesenchymal stem cells accelerate early remodeling of tendon-bone healing. *Cell Tissue Res* 2008; **332**: 469-478 [PMID: 18418628 DOI: 10.1007/s00441-008-0610-z]

100   **Utsunomiya H**, Uchida S, Sekiya I, Sakai A, Moridera K, Nakamura T. Isolation and characterization of human mesenchymal stem cells derived from shoulder tissues involved in rotator cuff tears. *Am J Sports Med* 2013; **41**: 657-668 [PMID: 23371475 DOI: 10.1177/0363546512473269]

101   **Kuhbier JW**, Weyand B, Sorg H, Radtke C, Vogt PM, Reimers K. [Stem cells from fatty tissue : A new resource for regenerative medicine? *Chirurg* 2010; **81**: 826-832 [PMID: 20830547 DOI: 10.1007/s00104-010-1962-y]

102   **García-Olmo D**, García-Arranz M, García LG, Cuellar ES, Blanco IF, Prianes LA, Montes JA, Pinto FL, Marcos DH, García-Sancho L. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis* 2003; **18**: 451-454 [PMID: 12756590 DOI: 10.1007/s00384-003-0490-3]

103   **Gimble J**, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy* 2003; **5**: 362-369 [PMID: 14578098 DOI: 10.1080/14653240310003026]

104   **Guilak F**, Estes BT, Diekman BO, Moutos FT, Gimble JM. 2010 Nicolas Andry Award:

Multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin Orthop Relat Res* 2010; **468**: 2530-2540 [PMID: 20625952 DOI: 10.1007/s11999-010-1410-9]

105   **Oh JH**, Chung SW, Kim SH, Chung JY, Kim JY. 2013 Neer Award: Effect of the adipose-derived stem cell for the improvement of fatty degeneration and rotator cuff healing in rabbit model. *J Shoulder Elbow Surg* 2014; **23**: 445-455 [PMID: 24129058 DOI: 10.1016/j.jse.2013.07.054]

106   **Valencia Mora M**, Antuña Antuña S, García Arranz M, Carrascal MT, Barco R. Application of adipose tissue-derived stem cells in a rat rotator cuff repair model. *Injury* 2014; **45** Suppl 4: S22-S27 [PMID: 25384471 DOI: 10.1016/S0020-1383(14)70006-3]

107   **Barco R**, Encinas C, Valencia M, Carrascal MT, García-Arranz M, Antuña S. [Use of adipose-derived stem cells in an experimental rotator cuff fracture animal model]. *Rev Esp Cir Ortop Traumatol* 2015; **59**: 3-8 [PMID: 25242729 DOI: 10.1016/j.recot.2014.07.008]

108   **Kosaka M**, Nakase J, Hayashi K, Tsuchiya H. Adipose-Derived Regenerative Cells Promote Tendon-Bone Healing in a Rabbit Model. *Arthroscopy* 2016; **32**: 851-859 [PMID: 26790583 DOI: 10.1016/j.arthro.2015.10.012]

109   **Lipner J**, Shen H, Cavinatto L, Liu W, Havlioglu N, Xia Y, Galatz LM, Thomopoulos S. In Vivo Evaluation of Adipose-Derived Stromal Cells Delivered with a Nanofiber Scaffold for Tendon-to-Bone Repair. *Tissue Eng Part A* 2015; **21**: 2766-2774 [PMID: 26414599 DOI: 10.1089/ten.TEA.2015.0101]

110   **Rothrauff BB**, Smith CA, Ferrer GA, Novaretti JV, Pauyo T, Chao T, Hirsch D, Beaudry MF, Herbst E, Tuan RS, Debski RE, Musahl V. The effect of adipose-derived stem cells on enthesis healing after repair of acute and chronic massive rotator cuff tears in rats. *J Shoulder Elbow Surg* 2019; **28**: 654-664 [PMID: 30527883 DOI: 10.1016/j.jse.2018.08.044]

111   **Kaizawa Y**, Franklin A, Leyden J, Behn AW, Tulu US, Sotelo Leon D, Wang Z, Abrams GD, Chang J, Fox PM. Augmentation of chronic rotator cuff healing using adipose-derived stem cell-seeded human tendon-derived hydrogel. *J Orthop Res* 2019; **37**: 877-886 [PMID: 30747435 DOI: 10.1002/jor.24250]

112   **Shin MJ**, Shim IK, Kim DM, Choi JH, Lee YN, Jeon IH, Kim H, Park D, Kholinne E, Yang HS, Koh KH. Engineered Cell Sheets for the Effective Delivery of Adipose-Derived Stem Cells for Tendon-to-Bone Healing. *Am J Sports Med* 2020; **48**: 3347-3358 [PMID: 33136454 DOI: 10.1177/0363546520964445]

113   **Gimble JM**, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007; **100**: 1249-1260 [PMID: 17495232 DOI: 10.1161/01.RES.0000265074.83288.09]

114   **Kim YS**, Sung CH, Chung SH, Kwak SJ, Koh YG. Does an Injection of Adipose-Derived Mesenchymal Stem Cells Loaded in Fibrin Glue Influence Rotator Cuff Repair Outcomes? *Am J Sports Med* 2017; **45**: 2010-2018 [PMID: 28448728 DOI: 10.1177/0363546517702863]

115   **Alentorn-Geli E**, Seijas R, Martínez-De la Torre A, Cuscó X, Steinbacher G, Álvarez-Díaz P, Barastegui D, Navarro J, Serra-Renom JM, Nishishinya B, Català J, Laiz P, García-Balletbó M, Cugat R. Effects of autologous adipose-derived regenerative stem cells administered at the time of anterior cruciate ligament reconstruction on knee function and graft healing. *J Orthop Surg (Hong Kong)* 2019; **27**: 2309499019867580 [PMID: 31470759 DOI: 10.1177/2309499019867580]

116   **Hopper RA**, Zhang JR, Fourasier VL, Morova-Protzner I, Protzner KF, Pang CY, Forrest CR. Effect of isolation of periosteum and dura on the healing of rabbit calvarial inlay bone grafts. *Plast Reconstr Surg* 2001; **107**: 454-462 [PMID: 11214061 DOI: 10.1097/00006534-200102000-00022]

117   **Ritsilä VA**, Santavirta S, Alhopuro S, Poussa M, Jaroma H, Rubak JM, Eskola A, Hoikka V, Snellman O, Osterman K. Periosteal and perichondral grafting in reconstructive surgery. *Clin Orthop Relat Res* 1994; 259-265 [PMID: 8168311]

118   **Burman MS**, Umansky M. An experimental study of free periosteal transplants, wrapped around tendon. *J Bone Joint Surg* 1930; 579-594 [DOI: 10.1002/bjs.1800176721]

119   **Karaoglu S**, Celik C, Korkusuz P. The effects of bone marrow or periosteum on tendon-to-bone tunnel healing in a rabbit model. *Knee Surg Sports Traumatol Arthrosc* 2009; **17**: 170-178 [PMID: 18941736 DOI: 10.1007/s00167-008-0646-3]

120   **Youn I**, Jones DG, Andrews PJ, Cook MP, Suh JK. Periosteal augmentation of a tendon graft improves tendon healing in the bone tunnel. *Clin Orthop Relat Res* 2004; 223-231 [PMID: 15021159 DOI: 10.1097/00003086-200402000-00037]

121   **Chen CH**, Chen WJ, Shih CH, Yang CY, Liu SJ, Lin PY. Enveloping the tendon graft with periosteum to enhance tendon-bone healing in a bone tunnel: A biomechanical and histologic study in rabbits. *Arthroscopy* 2003; **19**: 290-296 [PMID: 12627154 DOI: 10.1053/jars.2003.50014]

122   **Chang CH**, Chen CH, Su CY, Liu HT, Yu CM. Rotator cuff repair with periosteum for enhancing tendon-bone healing: a biomechanical and histological study in rabbits. *Knee Surg Sports Traumatol Arthrosc* 2009; **17**: 1447-1453 [PMID: 19440695 DOI: 10.1007/s00167-009-0809-x]

123   **Chen CH**, Liu HW, Tsai CL, Yu CM, Lin IH, Hsiue GH. Photoencapsulation of bone morphogenetic protein-2 and periosteal progenitor cells improve tendon graft healing in a bone tunnel. *Am J Sports Med* 2008; **36**: 461-473 [PMID: 18216273 DOI: 10.1177/0363546507311098]

124   **Chen CH**, Chang CH, Wang KC, Su CI, Liu HT, Yu CM, Wong CB, Wang IC, Whu SW, Liu HW. Enhancement of rotator cuff tendon-bone healing with injectable periosteum progenitor cells-BMP-2 hydrogel in vivo. *Knee Surg Sports Traumatol Arthrosc* 2011; **19**: 1597-1607 [PMID: 21327764 DOI: 10.1007/s00167-010-1373-0]

125   **Chang CH**, Chen CH, Liu HW, Whu SW, Chen SH, Tsai CL, Hsiue GH. Bioengineered periosteal progenitor cell sheets to enhance tendon-bone healing in a bone tunnel. *Biomed J* 2012; **35**: 473-480

[PMID: 23442360 DOI: 10.4103/2319-4170.104412]

126   **Chen L**, Whu S W, Chen C, Liu H, Chang C, Tsai C, Chen S, Hsiue G. Single-layer periosteum progenitor cell sheet significantly promotes tendon–bone healing in comparison with acellular collagen sheet in anterior cruciate ligament reconstruction. *Formos J Musculoskel Dis* 2012; **3**: 1-8 [DOI: 10.1016/j.fjmd.2012.01.001]

127   **Chen CH**, Chen WJ, Shih CH, Chou SW. Arthroscopic anterior cruciate ligament reconstruction with periosteum-enveloping hamstring tendon graft. *Knee Surg Sports Traumatol Arthrosc* 2004; **12**: 398-405 [PMID: 15060762 DOI: 10.1007/s00167-004-0498-4]

128   **Chen CH**, Chang CH, Su CI, Wang KC, Liu HT, Yu CM, Wong CB, Wang IC. Arthroscopic single-bundle anterior cruciate ligament reconstruction with periosteum-enveloping hamstring tendon graft: clinical outcome at 2 to 7 years. *Arthroscopy* 2010; **26**: 907-917 [PMID: 20620790 DOI: 10.1016/j.arthro.2009.11.011]

129   **Scheibel M**, Brown A, Woertler K, Imhoff AB. Preliminary results after rotator cuff reconstruction augmented with an autologous periosteal flap. *Knee Surg Sports Traumatol Arthrosc* 2007; **15**: 305-314 [PMID: 16927071 DOI: 10.1007/s00167-006-0173-z]

130   **Matsumoto T**, Ingham SM, Mifune Y, Osawa A, Logar A, Usas A, Kuroda R, Kurosaka M, Fu FH, Huard J. Isolation and characterization of human anterior cruciate ligament-derived vascular stem cells. *Stem Cells Dev* 2012; **21**: 859-872 [PMID: 21732814 DOI: 10.1089/scd.2010.0528]

131   **Matsumoto T**, Kubo S, Sasaki K, Kawakami Y, Oka S, Sasaki H, Takayama K, Tei K, Matsushita T, Mifune Y, Kurosaka M, Kuroda R. Acceleration of tendon-bone healing of anterior cruciate ligament graft using autologous ruptured tissue. *Am J Sports Med* 2012; **40**: 1296-1302 [PMID: 22427618 DOI: 10.1177/0363546512439026]

132   **Mifune Y**, Matsumoto T, Ota S, Nishimori M, Usas A, Kopf S, Kuroda R, Kurosaka M, Fu FH, Huard J. Therapeutic potential of anterior cruciate ligament-derived stem cells for anterior cruciate ligament reconstruction. *Cell Transplant* 2012; **21**: 1651-1665 [PMID: 22732227 DOI: 10.3727/096368912X647234]

133   **Mifune Y**, Matsumoto T, Takayama K, Terada S, Sekiya N, Kuroda R, Kurosaka M, Fu FH, Huard J. Tendon graft revitalization using adult anterior cruciate ligament (ACL)-derived CD34+ cell sheets for ACL reconstruction. *Biomaterials* 2013; **34**: 5476-5487 [PMID: 23632324 DOI: 10.1016/j.biomaterials.2013.04.013]

134   **Nakano N**, Matsumoto T, Takayama K, Matsushita T, Araki D, Uefuji A, Nagai K, Zhang S, Inokuchi T, Nishida K, Kuroda R, Kurosaka M. Age-dependent healing potential of anterior cruciate ligament remnant-derived cells. *Am J Sports Med* 2015; **43**: 700-708 [PMID: 25556219 DOI: 10.1177/0363546514561436]

135   **Inokuchi T**, Matsumoto T, Takayama K, Nakano N, Zhang S, Araki D, Matsushita T, Kuroda R. Influence of the Injury-to-Surgery Interval on the Healing Potential of Human Anterior Cruciate Ligament-Derived Cells. *Am J Sports Med* 2017; **45**: 1359-1369 [PMID: 28282242 DOI: 10.1177/0363546517689871]

136   **Takayama K**, Kawakami Y, Mifune Y, Matsumoto T, Tang Y, Cummins JH, Greco N, Kuroda R, Kurosaka M, Wang B, Fu FH, Huard J. The effect of blocking angiogenesis on anterior cruciate ligament healing following stem cell transplantation. *Biomaterials* 2015; **60**: 9-19 [PMID: 25965282 DOI: 10.1016/j.biomaterials.2015.03.036]

137   **Kawakami Y**, Takayama K, Matsumoto T, Tang Y, Wang B, Mifune Y, Cummins JH, Warth RJ, Kuroda R, Kurosaka M, Fu FH, Huard J. Anterior Cruciate Ligament-Derived Stem Cells Transduced With BMP2 Accelerate Graft-Bone Integration After ACL Reconstruction. *Am J Sports Med* 2017; **45**: 584-597 [PMID: 27903590 DOI: 10.1177/0363546516671707]

138   **Deans RJ**, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000; **28**: 875-884 [PMID: 10989188 DOI: 10.1016/s0301-472x(00)00482-3]

139   **Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147 [PMID: 10102814 DOI: 10.1126/science.284.5411.143]