

Review article

Biological fixation in anterior cruciate ligament surgery

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Received 23 December 2013; revised 12 February 2014; accepted 12 February 2014

Available online 29 March 2014

Abstract

Successful anterior cruciate ligament (ACL) reconstruction with tendon graft requires extensive tendon-to-bone healing in the bone tunnels and progressive graft ligamentization for biological, structural, and functional recovery of the ACL. Improvement in graft-to-bone healing is crucial for facilitating early, aggressive rehabilitation after surgery to ensure an early return to pre-injury activity levels. The use of various biomaterials for enhancing the healing of tendon grafts in bone tunnels has been developed. With the biological enhancement of tendon-to-bone healing, biological fixation of the tendon graft in the tunnel can be achieved in ACL reconstruction.

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Keywords: Anterior cruciate ligament; Biological fixation; Biomaterial; Graft healing; Tendon graft

Introduction

An anterior cruciate ligament (ACL) injury is a common knee injury among people of all ages, particularly athletes. The healing potential of a ruptured ACL is often extremely poor. In most cases, spontaneous ACL healing is insufficient. Reconstruction of the ACL is often required and has become a routine arthroscopic surgical procedure. The use of the semitendinosus and gracilis tendons in ACL reconstruction has become popular in recent years. However, successful ACL reconstruction with tendon grafting requires effective healing and integration of the tendon graft into the bone tunnels of the femur and tibia.

The native tendon or ligament insertion into bone is a highly specialized tissue that functions to transfer complex mechanical loads from soft tissue to bone. Ligaments have either direct or indirect insertion sites. The ACL inserts into bone through a direct insertion site that forms a transition zone from the tendon to the bone. This transition zone consists of the tendon, non-mineralized fibrocartilage, mineralized fibrocartilage, and

bone zones. Cartilage-specific collagens, including types II, IX, X, and XI, are found in the fibrocartilage of the insertion site, with collagen X playing a fundamental role in maintaining the interface between mineralized and demineralized fibrocartilage.^{1,2} Indirect insertion sites are composed of a type of collagen fibre known as Sharpey fibre that forms oblique to the long axis of the bone. The Sharpey fibres anchor the ligament to the bone, giving it mechanical strength.

The structure and composition of a healthy direct ACL insertion site is not reproduced after ligament reconstruction with tendon grafts. Studies have shown that, instead of regenerating the four zones of the native direct insertion site, the graft heals with an interposed layer of fibrovascular scar tissue at the graft–tunnel interface.^{3–6} Tendon-to-bone healing in a bone tunnel develops through bone ingrowth into the tissue of the fibrovascular interface that initially forms between the tendon and the bone. Progressive mineralization of the interface tissue occurs, with subsequent bone ingrowth into the outer tendon and the incorporation of the tendon graft into the surrounding bone. Progressive re-establishment of the continuity of the collagen fibres of the tendon and the bone results in the reformation of the tendon–bone junction.

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After ACL reconstruction, the tendon–bone interface initially consists of a woven bone formation that is fairly weak. The interface between the tendon graft and the bone tunnel contains three distinct histological zones that resemble a fibrous tendon insertion and the weak connection between the graft and bone tunnel may fail following ACL reconstruction.^{7–13} The firm attachment of the tendon graft to the bone is a critical factor in facilitating early aggressive rehabilitation and thus a speedier return to sports and work activities. Biological fixation initiates the graft to bone tunnel healing to achieve graft fixation in ACL reconstruction. Biological fixation techniques include intraoperative intervention with biological agents or postoperative extracorporeal intervention to enhance the graft–bone healing to achieve the effect of graft fixation and healing.

Many materials have been used to augment or enhance the healing of the tendon to the bone. Several studies have investigated strategies to improve bone ingrowth into tendon grafts. Autografts consisting of autologous ACL rupture tissues have been used to enhance tendon-to-bone healing.^{14–16} Treatments using osteoinductive factors, such as demineralized bone matrix and enamel matrix derivatives have enhanced tendon-to-bone healing in animal studies.^{17–19} Other osteoconductive materials may also improve tendon healing in the bone tunnel. Osteoconductive agents, such as calcium phosphate (CaP), hydroxyapatite, nanohydroxyapatite-based bone–graft substitute, tricalcium phosphate, brushite CaP cement, and CaP cement, have been used to improve bone formation around the tendon grafts.^{20–27} With the biological enhancement of tendon–bone healing, biological fixation of the tendon graft in the tunnel in the ACL reconstruction can be achieved.

Osteoclast-manipulation agents, such as alendronate and osteoprotegerin, have been used to inhibit osteoclast activity and promote bone formation at the tendon–bone interface.^{28,29} Stem cells are undifferentiated cells that, when directed by appropriate developmental signalling, can differentiate into a wide range of specialized cell types, thus representing a potential repair system for an equally wide range of tissues. Previous studies have evaluated the effects of stem cells from various sources on tendon-to-bone healing, including bone marrow stromal cells, mesenchymal stem cells, synovium-derived stem cells, and ACL-derived CD34+ cell sheets.^{30–37}

Histological and biomechanical studies have shown that growth factors, such as platelet-derived growth factor-BB, vascular endothelial growth factor, platelet-rich plasma (PRP), transforming growth factor, bone morphogenetic protein (BMP)-2, and granulocyte colony-stimulating factor, may improve tendon-to-bone healing.^{38–45} Indirect stimulation using low-intensity pulsed ultrasound, hyperbaric oxygen, or shock waves has been used for extracorporeal stimulation to enhance tendon-to-bone healing.^{46–48}

Simvastatin, an inhibitor of the competitive 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, has been shown to exert an anabolic effect on bone formation both *in vitro* and *in vivo* by activating the expression of BMP-2.⁴⁹ Using a rabbit model, a previous study showed that the local administration

of low-dose simvastatin-conjugated gelatin hydrogel promoted tendon-to-bone healing during the early phase following ACL reconstruction through the effect of simvastatin on angiogenesis and osteogenesis, but did not affect the long-term biomechanical properties of the reconstruction.⁴⁹

Periosteum, periosteal progenitor cell hydrogel, or periosteal progenitor cell sheet have been applied to enhance tendon-to-bone healing to achieve the effect of biological fixation. The periosteum consists of multipotent mesodermal cells and has been shown to contain chondroprogenitor and osteoprogenitor cells, which have the capacity to form cartilage and bone, respectively.^{50–53} In a rabbit model of ACL reconstruction, the effects of using periosteum-enveloped tendon graft in the bone tunnel for better graft–bone healing was achieved.^{54,55}

Periosteal progenitor cells (PPC) have the potential to differentiate to osteoprogenitor and chondroprogenitor cells. PPCs can be used to enhance tendon-to-bone healing through the formation of interface fibrocartilage. A novel injectable hydrogel containing PPCs and BMP-2 was evaluated in a rabbit model of tendon-to-bone healing. Histological analysis showed interface fibrocartilage and newly formed bone. Biomechanical testing revealed a significantly higher maximum pullout strength and stiffness in the experimental group. The tendon–bone interface undergoes a gradual remodelling process during healing. BMP-2 and PPCs are powerful enhancers of tendon-to-bone healing in the rabbit model.^{56–62} In the ACL reconstruction model, ACL reconstruction using the flexor digitorum longus tendon was performed in rabbits. Histological analysis of the tendon–bone interface in the bone tunnels showed fibrocartilage formation in the tendon–bone junction. Biomechanical testing revealed a significantly higher maximal pullout load at all time points. The PPC–BMP-2 hydrogel was a powerful enhancer of tendon-to-bone healing through the *de novo* formation of fibrocartilage.^{63,64}

Bioengineered PPC sheets were evaluated for the enhancement of tendon-to-bone healing in the extra-articular bone tunnel using a rabbit model. The tendon graft was wrapped in PPC sheets and inserted into the bone tunnel of the tibia. Histological staining of the tendon–bone junction showed that the PPC sheets enhanced collagen and glycosaminoglycan deposition in the newly formed fibrocartilage. Mature fibrocartilage and dense collagen fibres formed at the tendon–bone interface and more chondrocytes had formed at the interface of the animals treated with the PPC sheets. Treatment using the PPC sheets resulted in the formation of fibrocartilage and bone regeneration around the tendon graft.⁶⁵ The bioengineered PPC sheets represent a novel, feasible therapeutic strategy for enhancing tendon-to-bone healing following ACL reconstruction.

Clinical application of biological agents for enhancement of graft fixation and healing

The effects of periosteum, nanohydroxyapatite-based bone graft substitute, platelet concentrate (PC), and CaP on tendon-

to-bone healing has been evaluated in patients after ACL reconstruction surgery (Table 1).

Periosteum

In a case-series study, we evaluated single-bundle ACL reconstruction with periosteum-enveloped hamstring tendon graft. From 2000 to 2005, four-strand periosteum-enveloped hamstring tendon grafts were used for single-bundle ACL reconstruction in 312 patients who underwent at least a 2-year follow-up. The clinical follow-up assessments included the Lysholm knee score, the International Knee Documentation Committee (IKDC) score, arthrometric laxity testing (MED-metric KT-1000 Arthrometer, San Diego, CA, USA), and thigh muscle assessment. Radiographs were also used to assess the widening of the bone tunnels of the femur and tibia.

The mean follow-up period was 4.6 years (range 2–7 years). The median Lysholm knee score prior to and after surgery was 56 points (range 40–70 points) and 95 points (range 60–100 points), respectively. After ACL reconstruction, 85% of the patients returned to moderate or strenuous activity. Grade 2 or higher ligament laxity was observed in

5.1% of the patients based on the anterior drawer test and 6.1% of the patients had a positive pivot shift. Complete range of motion was achieved in 88% of the patients and 93% of the patients had an IKDC score within or near the normal range. Satisfactory results were achieved using the periosteum-enveloped hamstring tendon graft in the single-bundle ACL reconstruction and minimal tunnel widening was observed. Bone tunnel enlargement >1 mm was identified in 5.4% of the femoral tunnels and in 6.1% of tibial tunnels, which is less than that reported in previous studies that used comparable fixation.^{66,67}

Nanohydroxyapatite-based bone-graft substitute

A prospective study was designed to clinically and radiologically evaluate the efficacy of nanohydroxyapatite bone-based grafts for facilitating healing after hamstring tendon ACL reconstruction. To the best of our knowledge, this is the first study to assess the efficacy of such a bone substitute in humans. Forty men with chronic ACL rupture underwent surgical reconstruction with four-strand semitendinosus and gracilis tendon autograft using a single-bundle technique. The Lysholm, Tegner, IKDC, and arthrometric-laxity clinical assessments showed no significant difference between the two groups. Radiological analysis at the short or midterm follow-up examinations showed significantly greater graft strength, improved graft–bone interface healing, and reduced bone oedema in the patients who received the nanohydroxyapatite treatment. However, no significant long-term difference was observed between the two groups. Thus the use of the nanohydroxyapatite bone substitute did not provide significant clinical improvement in knee stability or patient satisfaction.²⁷

Hybridizing calcium phosphate

A novel technique has been proposed to improve tendon-to-bone healing by hybridizing CaP to the tendon graft using an alternating soaking process. Sixty-four patients with unilateral ACL rupture underwent arthroscopically-assisted single-bundle ACL reconstruction using a four-strand semitendinosus tendon or four-strand semitendinosus and gracilis tendon autograft, EndoButton femoral fixation (Acufex Microsurgical, Mansfield, MA, USA), and screw washer tibial fixation. Patients were randomly selected to undergo the CaP or the conventional reconstruction using the closed envelope method and a transtibial tunnel approach. In the CaP group, the tendon graft was hybridized with the CaP at both ends of the graft. One surgeon performed all the reconstructions and was blinded to the type of graft used. The data regarding age at surgery, sex, injury to surgery interval, and associated meniscal injuries were similar between the two groups. The follow-up assessments for all patients used the same postoperative protocol.

The KT-1000 arthrometry data indicated that the average anterior tibial translation was significantly lower in the CaP group at the 1- and 2-year follow-up evaluations compared with that of the conventional reconstruction group. Two years

Table 1
Biological fixation agents and techniques for enhancement of tendon–bone healing in anterior cruciate ligament reconstruction.

Material	Enhancement agent	Clinical application and effect
Autograft	Periosteum	+/+
	Autologous rupture tissue	–
Osteoinductive factors	Demineralized bone matrix	–
	Enamel matrix derivative	–
Osteoclast manipulation agents	Alendronate	–
	Osteoprotegerin	–
Growth factors	Platelet-rich plasma	+/-
	Bone morphogenetic protein	–
	Transforming growth factor	–
	Bone morphogenetic protein 2	–
	Granulocyte colony-stimulating factor	–
	Collagen-platelet composite	–
	Platelet concentrate	+
Bioactive materials	Calcium phosphate	+/+
	Calcium phosphate cement	+/+
	Nanohydroxyapatite-based bone graft substitute	+/+
	Tricalcium phosphate	–
	Brushite calcium phosphate cement	–
Stem cells	Bone marrow stromal cells	–
	Mesenchymal stem cells	–
	Synovium-derived stem cell	–
	Periosteum progenitor cell hydrogel	–
	Periosteum progenitor cell sheet	–
	Anterior cruciate ligament-derived CD34+ cell sheets	–
Indirect stimulation	Low-intensity pulsed ultrasound	–
	Hyperbaric oxygen	–
	Shock wave	–

+/+ = clinical application with positive effect; +/- = clinical application without significant effect; – = no clinical application.

after surgery the Lysholm score was significantly higher in the CaP group than in the conventional reconstruction group. One year after surgery, the CaP-hybridized tendon grafts showed less enlargement of the anteroposterior diameter of the main joint aperture site of the bone tunnel compared with that of the conventional reconstruction group. The results of the pivot-shift test, the IKDC assessment, the Tegner score, the intensity of the tendon graft in magnetic resonance imaging (MRI), and arthroscopic assessments were not significantly different between the two groups at both the 1- and 2-year follow-up examinations. The use of the CaP-hybridized tendon graft improved anterior knee stability and the Lysholm score at the 2-year follow-up. Less enlargement in both tunnels was also observed at the 1-year follow-up in the patients who received the CaP-hybridized graft compared with that in the patients who underwent conventional single-bundle ACL reconstruction.²⁵

Platelet concentrate

A prospective clinical study evaluated the use of PC for accelerating the healing process following ACL reconstruction. Patients requiring ACL reconstruction were randomly assigned to control, PC, bone plugs (BP), or combination PC and BP study groups. Maturation of the graft was evaluated at the femoral tunnel by using MRI maturation criteria based on a low-intensity signal, the absence of an osteoligamentous interface, and no widening of the femoral tunnel. Three months after surgery, no significant difference in MRI maturation was observed among the groups. Six months after the operation, 78% of the patients in the control group showed a low-intensity signal, whereas the low-intensity signal was present in 100% of the PC patients. No statistical difference in the osteoligamentous interface was observed between the various groups. Tunnel widening had occurred in 11% of the BP patients and 41% of the control patients, whereas no significant widening was observed among the other groups. The use of the PC enhanced graft maturation based on MRI signal intensity, but showed no significant effect on the osteoligamentous interface or the widening of the bone tunnel.¹⁵

Platelet-rich plasma

In a prospective clinical study of the use of PRP to enhance tendon-to-bone healing following ACL reconstruction, 40 patients were sequentially enrolled in Group A, Group B, Group C, or Group D. Group A received no PRP and Group B received PRP in the femoral tunnel at the end of the operation. Group C received PRP in the femoral tunnel at the end of the operation and intra-articularly at 2 weeks and 4 weeks after the operation. Group D received thrombin-activated PRP in the femoral tunnel. Three months after the operation, all patients underwent MRI of the knee to evaluate the signal intensity of the fibrous interzone in the femoral tunnels. No significant difference in signal intensity was observed among the various study groups.⁴³

Conclusion

Improvement in graft-to-bone healing is crucial to ensure early aggressive rehabilitation and an early return to pre-injury levels of physical activity. Tendon-to-bone healing with biological agents in a bone tunnel has been applied to achieve the effects of biological fixation in ACL reconstruction. Various biological agents such as growth factors and stem cells, including transforming growth factor β , BMP-2, granulocyte colony-stimulating factor, mesenchymal stem cells, ACL-derived CD34+ cell sheets, synovium-derived stem cells, PPC hydrogel, and PPC sheet, can play critical roles in tissue repair in both *in vitro* and *in vivo* studies. However, critical obstacles remain regarding the clinical application of these materials, such as dose determination, delivery technique, maintenance of the effect in the tunnel, and cost effectiveness. A simple, more reliable technique is required.

The application of certain biological agents to the tendon–bone interface and the bone tunnel have been evaluated in animal studies. These include the use of CaP, brushite CaP cement, demineralized bone matrix, magnesium-based bone adhesive, nanohydroxyapatite-based bone graft substitute, simvastatin-conjugated gelatin hydrogel, and enamel matrix derivative. However, the lack of extensive clinical evaluation of these materials limits their application.

The effects of periosteum, PC, PRP, CaP, and nanohydroxyapatite bone-base bone graft substitutes on tendon-to-bone healing following ACL reconstruction should be extensively evaluated clinically to clarify the effects of biological fixation. Indirect stimulation postoperatively using hyperbaric oxygen, low-intensity pulsed ultrasound, or shock waves have also been applied, but the effects of these techniques on tendon-to-bone healing also lack extensive clinical assessments.

Conflicts of interest

All authors declare no conflicts of interest.

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