Strategies to Enhance Tendon Graft - Bone Healing in Anterior Cruciate Ligament Reconstruction

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Tendon-bone incorporation of a tendon graft within the bone tunnel is a major concern when using a tendon graft for ligament reconstruction. Successful anterior cruciate ligament (ACL) reconstruction with a tendon graft requires solid healing of the tendon graft in the bone tunnels. Improvement of graft healing to bone is crucial to facilitate early and aggressive rehabilitation and a rapid return to full activity. Healing of a tendon graft in a bone tunnel requires bone ingrowth into the tendon. Indirect Sharpey fiber and direct fibrocartilage fixation of the tendon-bone interface provide different anchorage strength and interface properties. Based on normal ACL structure and the function of the insertion site, the ideal tendon graft would attach broadly to the surface of the bone at the femoral and tibial attachment sites by an intermediate zone of fibrocartilage. Theoretically, interface fibrocartilage formation as the translational structure from ligament to bone is physiological and functional. Our strategies to enhance tendon graft to bone healing, including the use of periosteum and a hydrogel containing periosteal progenitor cell and bone morphogenetic protein-2, are described. For clinical application, satisfactory results for ACL reconstruction can be achieved with the use of a periosteum-enveloped hamstring tendon graft. (Chang Gung Med J 2009;32:483-93)

Key words: arthroscopy, anterior cruciate ligament, tendon graft, tendon-bone healing

The healing potential of a ruptured anterior cruciate ligament (ACL) is considered to be extremely poor. ACL reconstruction using semitendinosus and gracilis tendons has become popular in recent years. However, ACL reconstruction requires that the tendon grafts heal in a surgically created bone tunnel. The primary site of weakness during the early postoperative period is the tendon-bone interface, particularly while the tendon heals to the bone within the intra-articular environment. The osteointegration of tendon grafts used for replacement of an ACL may still be unsatisfactory and may be associated with postoperative anterior-posterior laxity. Firm attachment of the tendon graft to the bone allows earlier and more aggressive rehabilitation and a quicker return to sports and work. Successful ACL reconstruction with a tendon graft necessitates effective healing of the tendon graft in the femoral and tibial bone tunnels. Tendon-bone healing in a bone tunnel occurs by bone ingrowth into the fibrovascu-
lar interface tissue that initially forms between the tendon and bone. Progressive mineralization of the interface tissue occurs first, with subsequent bone ingrowth into the outer tendon and incorporation of the tendon graft into the surrounding bone. Progressive reestablishment of the continuity of collagen fibers between the tendon and the bone results in re-establishment of a tendo-osseous junction. To improve ACL graft healing, new biological strategies to promote intra-articular and intraosseous healing are evolving. Although these biological engineering strategies are currently experimental, they are expected to be used in clinical application in the near future. Tendon graft to bone healing could be improved with the use of brushite calcium phosphate cement, injectable tricalcium phosphate, mesenchymal stem cells, hyperbaric oxygen treatment, transforming growth factor-beta 1 (TGF-β1), calcium-phosphate, bone marrow, demineralized bone matrix, synovial mesenchymal stem cells, granulocyte colony-stimulating factor, magnesium-based bone adhesive, bone morphogenetic protein-2 (BMP-2), low-intensity pulsed ultrasound, and shock wave therapy.

Strategies to improve tendon-bone tunnel healing have focused on providing appropriate molecular signals and cell differentiation resulting in an effective healing response between tendon and bone. A sufficient population of stem cells is likely required for optimal tissue regeneration. Mesenchymal stem cell-treated grafts have cartilage at the tendon-bone interface. Bone ingrowth plays an important role in graft-to-bone fixation. Several strategies have been demonstrated to improve bone ingrowth into a tendon graft placed in a bone tunnel. Most of these involve the use of osteoinductive cytokines. Hyperbaric oxygen, low-intensity pulsed ultrasound and extracorporeal shockwaves could induce marked increases in vascularity which improve new bone formation. Osteoconductive materials may also play a role in improving tendon healing in a bone tunnel via enriched bone ingrowth. Shock wave treatment significantly improves the healing rate of the tendon-bone interface with significantly more trabecular bone around the tendons. These various methods demonstrate the challenge of achieving secure biological fixation of tendon grafts in a bone tunnel with current ACL reconstruction techniques.

Our strategy to enhance tendon to bone healing is to use periosteum and hydrogel with periosteal progenitor cell-BMP-2.

The use of periosteum to enhance tendon-bone healing

Periosteum consists of multipotent mesodermal cells. It also contains chondroprogenitor and osteo-progenitor cells, which can form both cartilage and bone under appropriate conditions. Periosteum tissue may be used to improve the healing between tendon graft and bone. Our experimental studies have evaluated the effect of a periosteum-enveloping tendon graft on tendon-bone healing in 2 different experimental models in rabbits: a periosteum-enveloping tendon graft in a bone tunnel, and a periosteum-enveloping tendon graft in ACL reconstruction.

In the bone tunnel model, cross sections of the bone tunnel showed a fibrovascular interface tissue formed by periosteum tissue between the tendon and the bone. The cancellous bone lining in the bone tunnel with new bone formation was interdigitated with the fibrous interface tissue 4 weeks after the operation. The thickness of the interface fibrous layer surrounding the tendon was decreased. There was progressive mineralization and maturation of the new bone that grew into the interface fibrous layer. There appeared to be excellent integration between the fibrous interface layer and bone, and between the tendon and the interface layer at 8 weeks. The interface fibrous layer became integrated and mixed with the tendon and bone surface and no margin could be identified at 12 weeks. Progressive collagen fibrobone anchoring, maturation and organization between the tendon and bone lining developed. There was fibrocartilage formation appeared between the tendon and bone.

In the ACL reconstruction model, radiographs showed bone resorption as well as the formation of new bone around the femoral and tibial bone tunnels in the periosteum-treated tendon graft. There was further matrix deposition in the tendon-bone interface. There was variability in the degree of healing between the tendon and the bone in the control specimens, with some specimens demonstrating a persistent wide interface zone between the tendon and the bone, and only loose fibrous tissue had formed at 8 weeks. At 12 weeks after the operation, the perios-
teum-enveloping specimens demonstrated more cartilage and bone formation around the tendon graft in the femoral and tibial tunnels. There was extensive formation of new bone trabeculae and cartilage in the tendon-bone interface with new bone direct apposition to the tendon. A superior healing process and stronger healing strength could be achieved when periosteum was sutured on the tendon graft inserted into a bone tunnel.\(^{(33)}\)

This idea was used in ACL reconstructions to enhance healing of the tendon graft in the bone tunnels. The graft was composed of double loops of semitendinosus and gracilis tendon, 10 cm in length. A 3 x 3 cm periosteum flap was harvested from the anterior tibial cortex and divided to two 3 x 1.5 cm flaps. The periosteum was wrapped with the cambium layer placed outside to face the tunnel wall and then sutured on the tendon at both sides where the tendon graft approaches the tunnel opening.\(^{(34)}\) In the follow-up study, clinical assessments with the Lysholm knee score showed progression from 59 points before surgery to 94 points after surgery. After reconstruction, 81% of patients could return to moderate or strenuous activity and 94% of patients had normal or nearly normal ratings on International Knee Documentation Committee (IKDC) guidelines. Bone tunnel enlargement of more than 1 mm was identified in 5% of femoral tunnels and 6% of tibial tunnels. A satisfactory result can be achieved with the periosteum-enveloping hamstring tendon graft in ACL reconstruction. Periosteum can easily be harvested from the proximal tibia by a routine incision used to harvest hamstring tendons. In addition to its potential for improving tendon-bone healing, enveloped-periosteum may help to seal the intraarticular tunnel opening quickly after surgery, thus avoiding reflux of synovial fluid into the tunnel. Bone tunnel enlargement could be reduced (Fig. 1).\(^{(35)}\)

Fig. 1 ACL reconstruction with a periosteum-enveloping hamstring tendon autograft.
Injectable hydrogel with periosteal progenitor cells and bone morphogenetic protein-2 to enhance tendon graft-bone healing

Periosteal progenitor cells (PPC) have the potential to differentiate into osteogenic and chondrogenic cells in an adequate microenvironment. PPCs can be used to enhance the tendon-bone healing process by formation of interface fibrocartilage. A novel injectable hydrogel with PPCs and BMP-2 was created to enhance tendon-bone healing in a rabbit model.

Photopolymerization allows for an impressive degree of spatial and temporal control with implications for diverse, minimally invasive applications for tissue regeneration. Poly (ethylene glycol) diacrylate (PEGDA)-based polymers are frequently used in biomedical applications. Previous in vitro studies have indicated PEGDA-based polymers can provide a suitable microenvironment for growth and differentiation of mesenchymal stem cells. PPCs require appropriate signals to differentiate into cartilage and bone. BMP-induced signal transduction is an important positive regulator.

Several physiochemical aspects of hyaluronic acid (HA) are beneficial for biomaterial fabrication and application, and can transduce intrinsic signals within a structure, thereby enhancing tissue formation and playing a crucial role in promoting cell differentiation and cell growth. In our experimental studies, the effect of a hydrogel with PPCs and BMP-2 on tendon-bone healing was evaluated in 2 animal models, a tendon graft in a bone tunnel model and a tendon graft in an ACL reconstruction model.

In the bone tunnel model, the feasibility of using HA tethered to BMP-2 to stimulate PPCs to direct fibrocartilagenous attachment and new bone formation in an extra-articular tendon-bone healing model was examined in a rabbit model. The PPC-BMP-2 hydrogel was injected and photogelated in a bone tunnel. Histological analysis showed that interface fibrocartilage and new bone were formed by photoencapsulation of BMP-2 and PPCs at 6 weeks. Biomechanical testing revealed higher maximal pull-out strength and stiffness with a statistically significant difference from controls. The healing tendon-bone interface underwent a gradual remodeling process. It appears that photoencapsulation of BMP-2 and PPCs has a powerful inductive ability to induce healing between tendon and bone (Fig. 2).

In the ACL reconstruction model, the PPC-BMP-2 hydrogel was injected and photogelated in the femoral and tibial tunnel after ACL reconstruction with the flexor digitorum longus tendon in rabbits. Histological analysis of the tendon-bone interface in the femoral and tibial tunnels showed that an interface layer was formed by the hydrogel. At 4 weeks, there was fibrocartilage tissue in the focal area, and after 8 weeks, there was further matrix deposition with fibrocartilage formation in the tendon-bone junction. After 12 weeks, large areas of fibrocartilage at the tendon-bone junction could be detected. Biomechanical testing revealed higher maximal pull-out loads at all time points with statistically significant differences after 8 and 12 weeks between the treatment and control groups. The use of PEGDA-based hydrogel provided an adequate matrix for the encapsulation of cells and signal factors, and it was an effective local delivery method to reach the bone tunnel through injection. After the hydrogel is injected, it can be solidified via a photoinitiated polymerization process, which ensures encapsulation of the stem cells and growth factors. The PPC-BMP-2 hydrogel is a powerful inducer of tendon-bone healing through the neoformation of fibrocartilage (Fig. 3).

DISCUSSION

Tendon-bone incorporation of a tendon graft within a bone tunnel is a main concern when using a tendon graft for ligament reconstruction. Successful ACL reconstruction with a tendon graft requires solid healing of the tendon graft in the bone tunnels as soon as possible after surgery. Enhancing the healing of the tendon graft to bone is crucial to facilitate early and aggressive rehabilitation and a rapid return to full activity. The basic biology of tendon graft-bone tunnel healing remains incompletely understood. There is distinct variability in the morphological characteristics of the healing tendon-bone attachment site. Tendon-bone healing in a bone tunnel occurs via bone ingrowth into the fibrovascular interface tissue that forms between the tendon and bone. Previous studies have reported that the tendon-bone healing progresses via an interzone of vascular, highly cellular fibrous tissue, which undergoes a maturation process that lasts until its matrix consists of ori-
entated collagen fibers and the fibrous interface becomes indistinct. Progressive re-establishment of collagen fiber continuity between the tendon and bone facilitates the re-establishment of the tendo-osseous junction. The development of Sharpey-like collagen fibers that bridge the tendon graft and the bone has been described and is viewed as the earliest sign of osteointegration.

When a bone-patellar tendon-bone (BPTB) graft is used for ACL reconstruction, graft fixation depends on bone-to-bone healing. However, the length of the tendon portion of most BPTB grafts is greater than the intra-articular ACL length, resulting in the tendon portion in the tibial tunnel with tendon-to-bone healing rather than bone-to-bone healing. It is believed that graft fixation strength and healing are inferior for a tendon graft compared with a bone plug. In the BPTB graft, the bone plug is anchored with newly formed bone at 3 weeks. Degeneration of the tendon-bone junction in the plug progresses at 6 weeks. The weakest site differs not only between the 2 grafts but also between observation periods. In the tendon graft, the weakest site is the graft-wall interface at 3 weeks and the intraosseously grafted tendon at 6 weeks. In the BPTB graft, the weakest site is the graft-wall interface at 3 weeks and the proximal site in the bone plug at 6 weeks. The ultimate failure load of the tendon graft is significantly inferior to that of the BPTB graft at 3 weeks but there is no significant difference at 6 weeks.

There are spatial and temporal differences in tendon-to-bone healing at different regions of a surgically created bone tunnel. The healing tendon-bone interface tissue exhibits a wide chondroid matrix at

Fig. 2 Injectable hydrogel with PPCs and BMP-2 to enhance tendon graft-bone healing in a bone tunnel model.
the intra-articular aperture, in contrast to a narrow, fibrous matrix at the extra-articular aperture. Collagen continuity between the tendon graft and bone tunnel increases over time, with a more parallel orientation and increased collagen fiber continuity between the tendon and bone at the extra-articular aperture. Significant differences in healing between the tendon graft and bone exist along the length of the bone tunnel. The etiology of these differences is multifactorial, including variable biological and biomechanical environments at different sites in the tunnel.\(^{(57)}\)

On the basis of normal ACL structure and the known function of the insertion site, the ideal tendon graft would attach broadly to the surface of the bone at the femoral and tibial attachment sites by an intermediate zone of fibrocartilage.

Periosteum contains multipotent mesodermal cells, and an influence from the environment on the differentiation cells in free periosteal grafts has been shown.\(^{(34)}\) The periosteum has osteogenic capacity, and it can promote cartilage formation in a chondrotrrophic environment. Periosteum has the ability to initiate endochondral bone formation by inducing the differentiation of mesenchymal cells into chondroblasts and then subsequently into osteoblasts. Periosteum can also augment bone ingrowth into collagenous tissue and induce ossification and bone formation. Free autologous periosteal transplants have been reported to produce hyaline-like cartilage in chondral defects of the patella, which suggests the potential for stem cells in the cambium layer to produce cartilage.\(^{(58)}\) Thus, based on the findings of these studies, periosteum applied to the surface of a tendon graft should form cartilage or bone tissue. In addition, if the periosteum-enveloping tendon graft is placed in a tunnel extra-articularly or intra-articularly, it may promote initiation and regulation of bone ingrowth into the tendon graft.

When periosteum is sutured on the surface of the intra-articular aperture, in contrast to a narrow, fibrous matrix at the extra-articular aperture.

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Fig. 3 Injectable hydrogel with PPCs and BMP-2 to enhance tendon graft-bone healing in an ACL reconstruction model.
the tendon and then transplanted into a bone tunnel, the cambium layer serves as a fibrous layer between tendon and bone. When bone ingrowth into the cambium layer appears at 4 weeks, there is interdigitation between the periosteum tissue and tendon. This interface fibrous layer, which originates from the wrapped periosteum, shows progressive incorporation and organization of the interface developed over time.

In our study, there was progressive maturation of the tissue at the interface of the tendon and bone. There was extensive fibrocartilage formation in the tendon-bone interface. The periosteum has a powerful inductive ability to enhance healing between the tendon and the bone tunnel. Periosteum can induce differentiation of the mononuclear cells into chondroblastic and osteoblastic cells. Then, direct fibrocartilage or osteoid production, followed by mineralization and progressive remodeling, develops during the healing process.

Because the periosteum has a rapid effect on bone ingrowth with improved fixation strength, it is possible that enveloping the tendon graft with periosteum may be an effective way to avoid delayed graft healing. Periosteum may be even more effective in situations in which healing is impaired, such as in patients with a widened bone tunnel in a revision operation. Photopolymerizable hydrogel with PPC and BMP-2 would also enhance the formation of interpenetrating networks that may limit the tunnel enlargement and improve the healing response of the graft in the tunnel.

Tunnel expansion is significantly greater following ACL reconstruction using hamstring tendon autografts. This is because of the greater distance from the normal insertion site and the biomechanical point of action of the ACL. This creates a potentially larger force moment during graft cycling which may lead to greater expansion of the bone tunnels. Adequate earlier tendon-bone healing may solve this problem. This technique may be applied to ACL reconstruction to enhance tendon graft healing within the tunnel.

In order to enhance tendon-bone tunnel healing, our laboratory intended to develop an injectable hydrogel to fill the tendon-bone tunnel interface by a tissue engineering approach. Tissue engineering therapies for biomimetic material rely on the stimulation of signaling growth factor to induce cellular chemo- taxis, proliferation, differentiation, and induce notably new tissue formation at a required site. Recent studies have shown that various growth factors play important roles in tissue repair both in vivo and in vitro. However, there are several critical problems that must be addressed for the clinical application of this technique, such as dose determination, delivery technique, maintenance of the effect in the tunnel, and cost-effect issues. In addition, a simple and reliable technique is needed.

Photopolymerized hydrogels used as scaffolds in tissue-engineering techniques typically act as carriers or fillers in biological systems. Scaffolds should, in part, mimic the structure and biological function of an extracellular matrix. They should promote cell proliferation, induce cell differentiation, or enhance growth of surrounding tissues. Numerous biological signals have been used as functional additives in hydrogels to enhance the development of engineered tissues. Biological signals such as growth factors can be physically encapsulated in hydrogels along with cells. To prevent the loss of growth factors from hydrogels by leaching or extraction, growth factors can be covalently tethered to the hydrogel. This tethering offers prolonged retention, and relevant growth factor induction of signaling pathways. The combination of BMP-2 with a hydrogel for delivery to a target site is essential to allow BMP-2 activity. Photocapsulation of BMP-2 in a PEGDA-based hydrogel provides temporally and spatially defined modulation of chondroblastic and osteoblastic differentiation. Consistent stimulation by prolonged BMP-2 retention enhances the migration and proliferation of PPCs.

Adequate mechanical loading on the tendon graft should be one of the basic requirements for long-time survival of the graft tissue. Tendon healing in a bone tunnel is influenced by mechanical stress and it has been shown that the differentiation of mesenchymal stem cells is directly influenced by pressure and tension. In the bone tunnel, mechanical loading occurs mainly by shear forces, which might prevent or delay the development of a fibrocartilage zone, leading to the development of an indirect insertion.

Based on the finding that tendon-bone healing progresses by bone ingrowth into the fibrous tissue interzone, exogenous osteoinductive agents should be used to augment this healing. We have demon-
stratified improved healing by applying injectable photopolymerizable hydrogel at the tendon-bone interface. Our study suggests that photoencapsulation of BMP-2 and PPCs has the ability to improve healing between tendon and bone. This technique may provide a novel platform for tissue-engineered stem cell therapy.

Conclusions
Successful ACL reconstruction with a tendon graft requires solid healing of the tendon graft in the bone tunnels. Satisfactory results can be achieved with a periosteum-enveloping hamstring tendon graft in ACL reconstruction. PEGDA-based hydrogel provides an adequate matrix for the encapsulation of cells and signal factors. The hydrogel could be effectively delivered to the bone tunnel by injection. After injecting the hydrogel, it can be solidified with a photoinitiated polymerization process to assure encapsulation of the stem cells and growth factors. The PPC-BMP-2 hydrogel is a powerful inducer of healing between tendon and bone through the neof ormation of fibrocartilage.

REFERENCES


前十字韌帶重建術後促進肌腱移植物與骨頭癒合的策略

陳志華

成功的前十字韌帶重建術需要穩固的肌腱移植物與骨隧道的癒合。如何促進肌健移植物與骨頭的癒合是一個重要的課題。骨膜包含有多種潛在性的中胚層細胞並富含骨原及軟骨原細胞，具有形成骨頭及軟骨新生的能力。在動物實驗中，骨膜包裹肌健可以促進肌腱移植物在骨隧道的癒合。我們將此研究成果應用在臨床之前十字韌帶重建術上。在臨床追蹤研究中，可以得到滿意的成果。我們也發展了以光聚合膠作為骨膜骨原細胞與骨生成因子之載體而注射至骨隧道之技術，以促進肌腱移植物與骨頭的癒合。動物實驗顯示可以藉著纖維軟骨的生成，有效促進肌腱在骨隧道中之癒合。藉著新型水膠之組織工程技術可以將細胞治療適用於臨床十字韌帶重建中以促進癒合，使病人得以早日回復正常運動能力。(長庚醫誌 2009;32:483-93)

關鍵詞：關節鏡，前十字韌帶，肌腱移植物，肌腱骨頭癒合