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

Chih-Hwa Chen, MD

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)*

Dr. Chih-Hwa Chen

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.[1-3] 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-

From the Department of Orthopaedic Surgery, Chang Gung Memorial Hospital, Keelung, Chang Gung University College of Medicine, Taoyuan, Taiwan.
Received: Nov. 28, 2008; Accepted: Mar. 25, 2009
Correspondence to: Dr. Chih-Hwa Chen, Department of Orthopaedic Surgery, Chang Gung Memorial Hospital. 222, Maijin Rd., Anle District, Keelung City 204, Taiwan (R.O.C.) Tel.: 886-2-24313131 ext. 2613; Fax: 886-2-24332655;
E-mail: afachen@doctor.com

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.[4-10] 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.[11-26]

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.[13,16,18,19] 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 have involve the use of osteoinductive cytokines.[15,20,22-24] Hyperbaric oxygen, low-intensity pulsed ultrasound and extracorporeal shockwaves could induce marked increases in vascularity which improve new bone formation.[14,25,26] Osteoconductive materials may also play a role in improving tendon healing in a bone tunnel via enriched bone ingrowth.[11,12,17,21,25] Shock wave treatment significantly improves the healing rate of the tendon-bone interface with significantly more trabecular bone around the tendons.[26] 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 osteoprogenitor cells, which can form both cartilage and bone under appropriate conditions.[27-31] 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.[32,33]

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 fiber-bone anchoring, maturation and organization between the tendon and bone lining developed. There was fibrocartilage formation appeared between the tendon and bone.[32]

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]

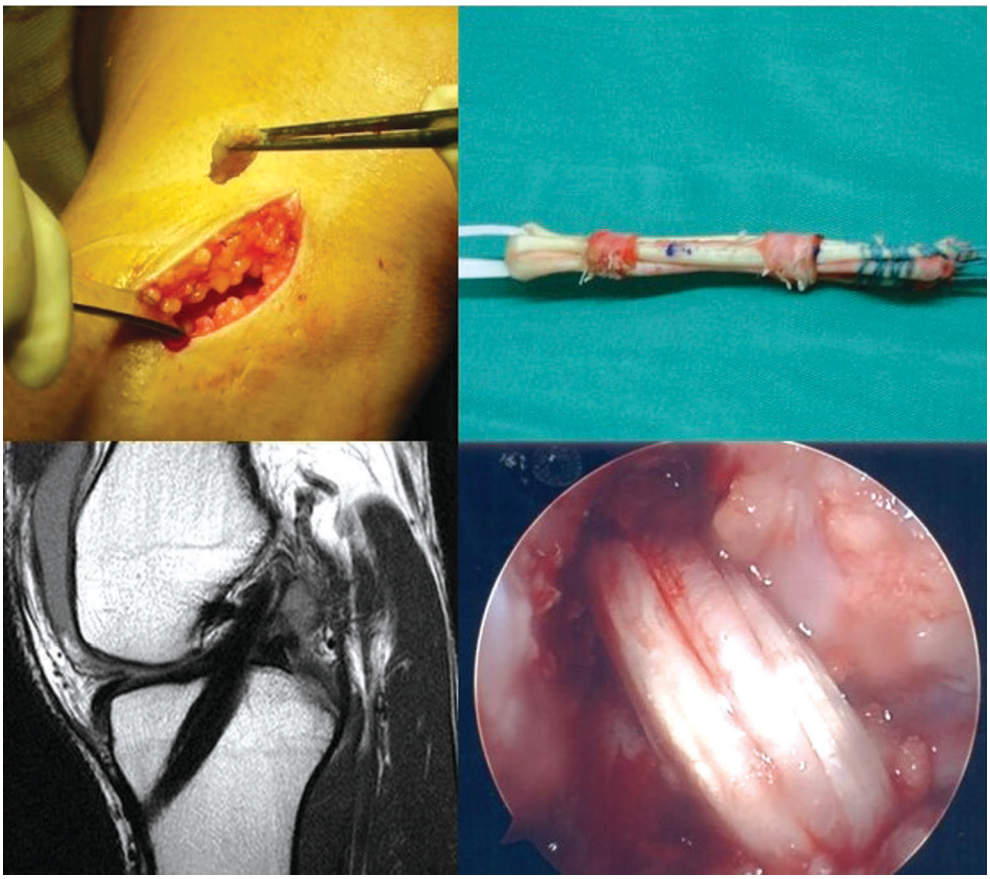

Fig. 1 ACL reconstruction with a periosteum-enveloping hamstring tendon autograft.

Injectable hydrogel with periosteal progenitor cells and bone morphogenic protein-2 to enhance tendon graft-bone healing

Periosteal progenitor cells (PPC) have the potential to differentiate into osteogenitor and chondrogenitor 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.[36-44] 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.[45] PPCs require appropriate signals to differentiate into cartilage and bone. BMP-induced signal transduction is an important positive regulator.[46-49]

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.[50,51] 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.[52,53]

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).[52]

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).[53]

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-

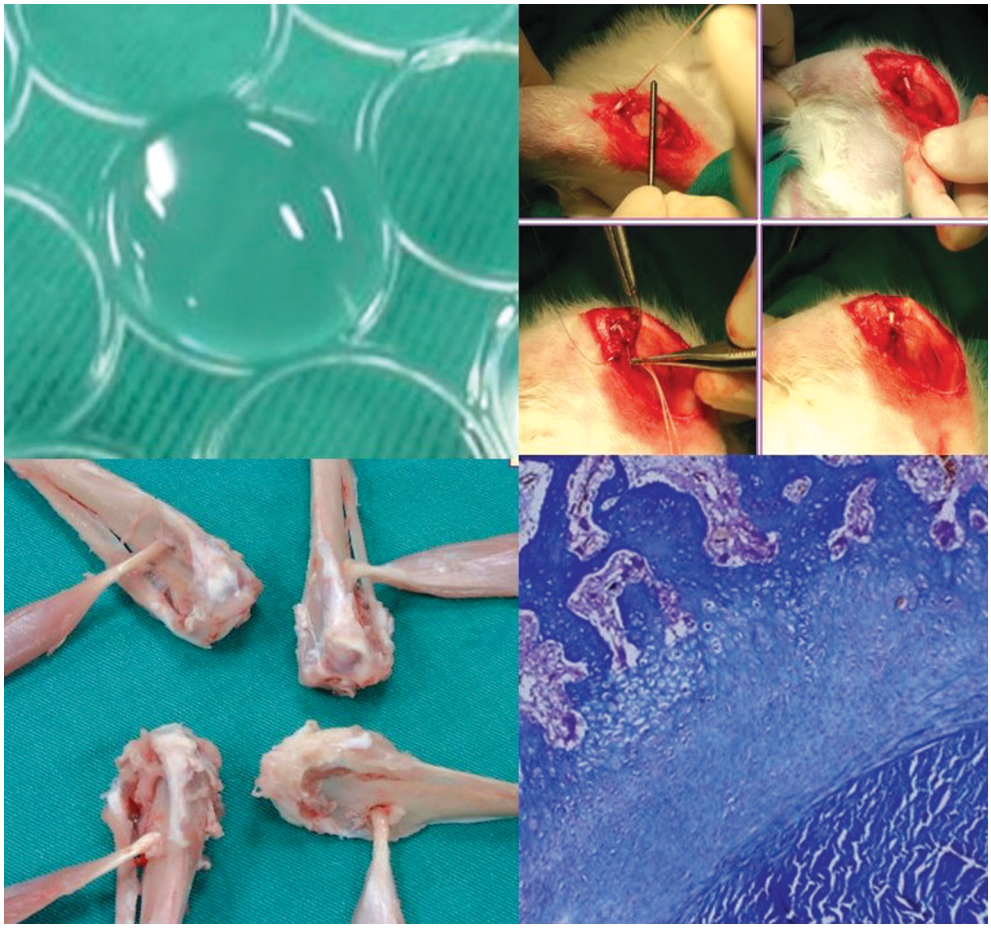

Fig. 2 Injectable hydrogel with PPCs and BMP-2 to enhance tendon graft-bone healing in a bone tunnel model.

entated collagen fibers and the fibrous interface becomes indistinct.[4,54] Progressive re-establishment of collagen fiber continuity between the tendon and bone facilitates the re-establishment of the tendo-osseous junction.[1] 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.[55]

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 s 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.[56]

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

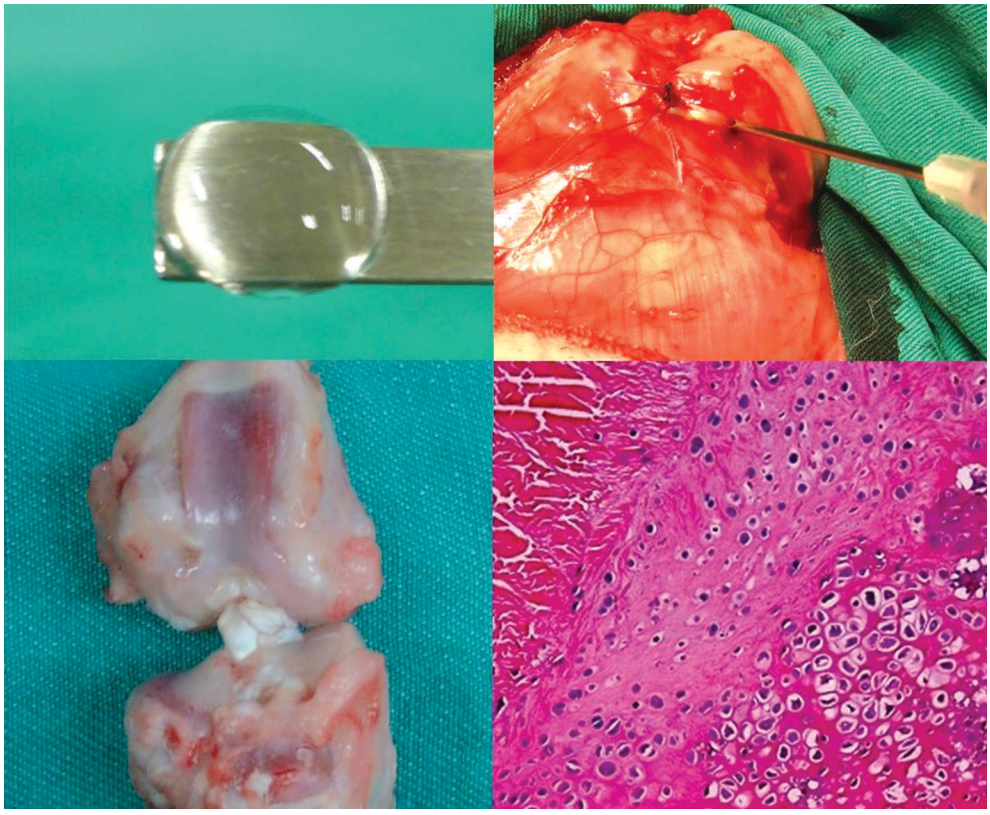

Fig. 3 Injectable hydrogel with PPCs and BMP-2 to enhance tendon graft-bone healing in an ACL reconstruction model.

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 chondrotrophic 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 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 developd 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.[59] 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 chemotaxis, 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.[11,55,60-65] 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.[51] To prevent the loss of growth factors from hydrogels by leaching or extraction, growth factors can be covalently tethered to the hydrogel.[66] 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. Photoencapsulation 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.[67] 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-

strated improved healing by applying injectable photopolymerizable hydrogel at the tendon-bone interface.[38,68] 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 neoformation of fibrocartilage.

REFERENCES

1. Beynnon BD, Johnson RJ, Abate JA, Fleming BC, Nichols CE. Treatment of anterior cruciate ligament injuries, part 2. Am J Sports Med 2005;33:1751-67.
2. Beynnon BD, Johnson RJ, Abate JA, Fleming BC, Nichols CE. Treatment of anterior cruciate ligament injuries, part I. Am J Sports Med 2005;33:1579-602.
3. Sundar S, Pendegrass CJ, Blunn GW. Tendon bone healing can be enhanced by demineralized bone matrix: A functional and histological study. J Biomed Mater Res B Appl Biomater 2009;88:115-22.
4. 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;75:1795-803.
5. Eriksson E. Vascular ingrowth into ACL-grafts. Knee Surg Sports Traumatol Arthrosc 2008;16:341.
6. Gulotta LV, Rodeo SA. Biology of autograft and allograft healing in anterior cruciate ligament reconstruction. Clin Sports Med 2007;26:509-24.
7. Nebelung W, Becker R, Urbach D, Röpke M, Roessner A. Histological findings of tendon-bone healing following anterior cruciate ligament reconstruction with hamstring grafts. Arch Orthop Trauma Surg 2003;123:158-63.
8. Weiler A, Hoffmann RF, Bail HJ, Rehm O, Südkamp NP. Tendon healing in a bone tunnel. Part II: Histologic analysis after biodegradable interference fit fixation in a model of anterior cruciate ligament reconstruction in sheep. Arthroscopy 2002;18:124-35.
9. Weiler A, Peine R, Pashmineh-Azar A, Abel C, Südkamp NP, Hoffmann RF. Tendon healing in a bone tunnel. Part I: Biomechanical results after biodegradable interference fit fixation in a model of anterior cruciate ligament reconstruction in sheep. Arthroscopy 2002;18:113-23.
10. Hays PL, Kawamura S, Deng XH, Dagher E, Mithoefer K, Ying L, Rodeo SA. The role of macrophages in early healing of a tendon graft in a bone tunnel. J Bone Joint Surg Am 2008;90:565-79.
11. Wen CY, Qin L, Lee KM, Chan KM. The use of brushite calcium phosphate cement for enhancement of bone-tendon integration in an anterior cruciate ligament reconstruction rabbit model. J Biomed Mater Res B Appl Biomater. 2008 Oct 20.
12. Huangfu X, Zhao J. Tendon-bone healing enhancement using injectable tricalcium phosphate in a dog anterior cruciate ligament reconstruction model. Arthroscopy 2007;23:455-62.
13. 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;35:962-71.
14. Yeh WL, Lin SS, Yuan LJ, Lee KF, Lee MY, Ueng SW. Effects of hyperbaric oxygen treatment on tendon graft and tendon-bone integration in bone tunnel: biochemical and histological analysis in rabbits. J Orthop Res 2007;25:636-45.
15. Yamazaki S, Yasuda K, Tomita F, Tohyama H, Minami A. The effect of transforming growth factor-beta1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. Arthroscopy 2005;21:1034-41.
16. 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.
17. Mutsuzaki H, Sakane M, Nakajima H, Ito A, Hattori S, Miyanaga Y, Ochiai N, Tanaka J. Calcium-phosphate-hybridized tendon directly promotes regeneration of tendon-bone insertion. J Biomed Mater Res A 2004;70:319-27.
18. 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;17:170-8.
19. 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;332:469-78.
20. Sasaki K, Kuroda R, Ishida K, Kubo S, Matsumoto T, Mifune Y, Kinoshita K, Tei K, Akisue T, Tabata Y, Kurosaka M. Enhancement of tendon-bone osteointegration of anterior cruciate ligament graft using granulocyte

colony-stimulating factor. Am J Sports Med 2008;36:1519-27.
21. Gulotta LV, Kovacevic D, Ying L, Ehteshami JR, Montgomery S, Rodeo SA. Augmentation of tendon-to-bone healing with a magnesium-based bone adhesive. Am J Sports Med 2008;36:1290-7.
22. 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;27:476-88.
23. Hashimoto Y, Yoshida G, Toyoda H, Takaoka K. Generation of tendon-to-bone interface "enthesis" with use of recombinant BMP-2 in a rabbit model. J Orthop Res 2007;25:1415-24.
24. Yu Y, Bliss JP, Bruce WJ, Walsh WR. Bone morphogenetic proteins and Smad expression in ovine tendon-bone healing. Arthroscopy 2007;23:205-10.
25. Walsh WR, Stephens P, Vizesi F, Bruce W, Huckle J, Yu Y. Effects of low-intensity pulsed ultrasound on tendon-bone healing in anintra-articular sheep knee model. Arthroscopy 2007;23:197-204.
26. Wang CJ, Wang FS, Yang KD, Weng LH, Sun YC, Yang YJ. The effect of shock wave treatment at the tendon-bone interface - A histomorphological and biomechanical study in rabbits. J Orthop Res 2005;23:274-80.
27. Breitbart AS, Grande DA, Kessler R, Ryaby JT, Fitzsimmons RJ, Grant RT. Tissue engineered bone repair of calvarial defects using cultured periosteal cells. Plast Reconstr Surg 1998;101:567-74.
28. Ritsila V, Alhopuro S, Rintala A. Bone formation with free periosteum. Scand J Plast Reconstr Surg 1972;6:51-6.
29. Rubak J NI. Osteochondrogenesis of free periosteal grafts in the rabbit iliac crest. Acta Orthop Scand 1983;54:826-31.
30. Uddstrdmer L, Ritsila V. Osteogenic capacity of periosteal grafts. Scand J Plast Reconstr Surg Hand Surg 1978;12:207-14.
31. Liu HW, Chen CH, Tsai CL, Hsiue GH. Targeted delivery system for juxtacrine signaling growth factor based on rhBMP-2-mediated carrier-protein conjugation. Bone 2006;39:825-36.
32. Chen CH, Chen WJ, Shih CH. Enveloping of periosteum on the tendon graft to enhance tendon-bone healing in a bone tunnel – A biomechanical and histological studies in rabbits. Arthroscopy 2003;19:290-6.
33. Chen CH, Chen LH, Chen WJ, Shih CH, Yang CY, Liu SJ, Lin PY. Periosteum-enveloping of the tendon to enhance tendon-bone healing in the bone tunnel – Histologic studies in three experimental rabbit models. J Orthop Surg Taiwan 2003;20:21-9.
34. Chen CH, Chen WJ, Shih CH. Enveloping of periosteum on the hamstring tendon graft in anterior cruciate ligament reconstruction. Arthroscopy 2002;18:e27-40.
35. 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.
36. Kernwein GA. A study of tendon implantations into bone. Surg Gynec Obstet 1942;75:794-6.
37. Sawhney AS, Pathak CP, van Rensburg JJ, Dunn RC, Hubbell JA. Optimization of photopolymerized bioerodible hydrogel properties for adhesion prevention. J Biomed Mater Res 1994;28:831-8.
38. An Y, Hubbell JA. Intraarterial protein delivery via intimally adherent bilayer hydrogels. J Control Release 2000;64:205-15.
39. Lu S, Ramirez WF, Anseth KS. Photopolymerized, multilaminated matrix devices with optimized nonuniform initial concentration profiles to control drug release. J Pharm Sci 2000;89:45-51.
40. Cruise GM, Hegre OD, Lamberti FV, Hager SR, Hill R, Scharp DS, Hubbell JA. In vitro and in vivo performance of porcine islets encapsulated in interfacially photopolymerized poly (ethylene glycol) diacrylate membranes. Cell Transplant 1999;8:293-306.
41. Elisseeff J, McIntosh W, Anseth K, Riley S, Ragan P, Langer R. Photoencapsulation of chondrocytes in poly (ethylene oxide)-based semi-interpenetrating networks. J Biomed Mater Res 2000;51:164-71.
42. Suggs LJ, Mikos AG. Development of poly (propylene fumarateco-ethylene glycol) as an injectable carrier for endothelial cells. Cell Transplant 1999;8:345-50.
43. Anseth KS, Metters AT, Brynt SJ, Martens PJ, Elisseeff JH, Bowman CN. In situ forming degradable networks and their application in tissue engineering and drug delivery. J Control Release 2003;78:199-209.
44. Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Transdermal photopolymerization for minimally invasive implantation. Proc Natl Acad Sci USA 1999;96:3104-7.
45. Williams CG, Kim TK, Taboas A, Malik A, Manson P, Elisseeff J. In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. Tissue Eng 2003;9:679-88.
46. de John DS, Steegenga WT, Hendriks JMA, van Zoelen EJJ, Olijve W, Dechering KJ. Regulation of notch signaling genes during BMP2-induced differentiation of osteoblast precursor cells. Biochem Biophys Res Commun 2004;320:100-7.
47. Kawabata M, Imamura T, Miyazono K. Signal transduction by bone mophogenetic proteins. Cytokine Growth Factor Rev 1998;9:49-61.
48. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA. Novel regulators of bone formation: molecular clones and activities. Science 1988;242:1528-34.
49. Thomopoulos S, Hattersley G, Rosen V, Mertens M, Galatz L, Williams GR, Soslowsky LJ. The localized expression of extracellular matrix components in healing tendon insertion sites: an in situ hybridization study. J Orthop Res 2002;20:454-63.
50. Laurent TC, Laurent UB, Fraser JR. The structure and

function of hyaluronan: an overview. Immunol Cell Biol 1996;74:A1-7.
51. Kawasaki K, Ochi M, Uchio Y, Adachi N, Matsusaki M. Hyaluronic acid enhances proliferation and chondroitin sulfate synthesis in cultured chondrocytes embedded in collagen gels. J Cell Physiol 1999;179:142-8.
52. 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;36:461-73.
53. Chen CH, Liu HW, Hsiue GH, Tsai CL, Yu CM, Lin IH. Photoencapsulation of bone morphogenetic protein-2 and periosteal progenitor cells enhance tendon-bone healing in anterior cruciate ligament reconstruction. Trans Orthop Res Soc 2008;33:255.
54. 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;22:344-51.
55. Liu S, Panossian V, Al-Shaikh R, Tomin E, Shepherd E, Finerman G, Lane J. Morphology and matrix composition during early tendon to bone healing. Clin Orthop 1997;339:253-60.
56. Tomita F, Yasuda K, Mikami S, Sakai T, Yamazaki S, Tohyama H. Comparisons of intraosseous graft healing between the doubled flexor tendon graft and the bone-patellar tendon-bone graft in anterior cruciate ligament reconstruction. Arthroscopy 2001;17:461-76.
57. Steiner ME, Murray MM, Rodeo SA. Strategies to improve anterior cruciate ligament healing and graft placement. Am J Sports Med 2008;36:176-89.
58. Ritsila 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 1994;302:259-65.
59. Fahey M, Indelicato PA. Bone tunnel enlargement after anterior cruciate ligament replacement. Am J Sports Med 1994;22:410-4.
60. Anderson K, Seneviratne AM, Izawa K, Atkinson BL, Potter HG, Rodeo SA. Augmentation of tendon healing in an intra-articular bone tunnel with use of a bone growth factor. Am J Sports Med 2001;29:689-98.
61. DesRosiers EA, Yahia L, Rivard CH. Proliferative and matrix synthesis response of canine anterior cruciate ligament fibroblasts submitted to combined growth factors. J Orthop Res 1996;14:200-8.
62. Kobayashi D, Kurosaka M, Yoshiya S, Mizuno K. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc 1997;5:189-94.
63. Martinek V, Latterman C, Usas A, Abramowitch S, Woo SL, Fu FH, Huard J. Enhancement of tendon-bone integration of anterior cruciate ligament grafts with bone morphogenetic protein-2 gene transfer: a histological and biomechanical study. J Bone Joint Surg Am 2002;84:1123-31.
64. Marui T, Niyibizi C, Georgescu HI, Cao M, Kavalkovich KW, Levine RE, Woo SL. Effect of growth factors on matrix synthesis by ligament fibroblasts. J Orthop Res 1997;15:18-23.
65. Spindler KP, Murray MM, Detwiler KB, Tarter JT, Dawson JM, Nanney LB, Davidson JM. The biomechanical response to doses of TGF-beta 2 in the healing rabbit medial collateral ligament. J Orthop Res 2003;21:245-9.
66. Mann BK, Schmedlen RH, West JL. Tethered-TGF-beta increases extracellular matrix production of vascular smooth muscle cells. Biomaterials 2001;22:439-44.
67. Yamakado K, Kitaoka K, Yamada H, Hashiba K, Nakamura R, Tomita K. The influence of mechanical stress on graft healing in a bone tunnel. Arthroscopy 2002;18:82-90.
68. Ohtera K, Yamada Y, Aoki M, Sasaki T, Yamakoshi K. Effect of periosteum wrapped around tendon in a bone tunnel: A biomechanical and histological study in rabbits. Crit Rev Biomed Eng 2000;28:115-8.

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。如何促進肌腱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，骨膜包裹肌腱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爲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運用在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

長庚紀念醫院 基隆院區 骨科；長庚大學 醫學院
受文日期：民國97年11月28日；接受刊載：民國98年3月25日
通訊作者：陳志華醫師，長庚紀念醫院 基隆院區 骨科。基隆市204安樂區麥金路222號。Tel.: (02)24313131轉2613; Fax: (02)24332655; E-mail: afachen@doctor.com