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Enhancement of Tendon to Bone Healing in ACL Reconstruction – Basic Research and Clinical Application

Department of Orthopaedic Surgery,
Chang Gung Memorial Hospital – Keelung, Chang Gung University, Taiwan

Chih-Hwa Chen, MD

ACL reconstruction with hamstring tendon is popular in recent years. Successful ACL reconstruction with tendon graft requires solid healing of the tendon graft in the bone tunnels. In the early healing period, the site of tendon graft attachment to bone indicates the weakest site, it is often necessary to delay mobilization, physical therapy, range of motion, and specific activities to protect the healing graft from excessive loads. Improvement and enhancement in graft healing to bone in the tunnels is critical to allow earlier and more aggressive rehabilitation and earlier return to work or sport.

Enhancement of tendon to bone healing in ACL reconstruction – basic research

1. Periosteum–enveloping tendon graft for tendon–bone healing in ACL reconstruction

The periosteum is another repository for mesenchymal stem cells. This cell layer clearly responds to injury by cellular expansion and can form woven bone quickly. The mesenchymal stem cells in the periosteum, like those in the marrow, are developmentally multipotential and able to form complex and different tissues of the mesodermal lineage. Numerous studies have also demonstrated the potential for periosteum to induce new bone formation. It is possible that periosteum tissue can be used to improve the healing process between tendon and bone. The cambium layer of the periosteum could serve as a potent interface layer that became progressive matured and organized during the healing process, resulting in close integration of the disrupted tendon to the bone. Adult new

Zealand white rabbits were used in the study. The long digitorum extensor tendon of hind limbs were used to be the tendon graft. In one limb, the periosteum from the proximal tibia was sutured on the surface of the tendon portion. The cambium side was at outside surface. The contralateral limb without periosteum-enveloping on the tendon was used as a control. The rabbits were sacrificed at 4, 8, 12 weeks after operation. Histological analysis of tendon-bone interface in the periosteum-enveloping group showed a layer of fibrous tissue between the tendon and the bone. This interface fibrous layer, original from wrapped periosteum, became matured and organized during the healing process. Evidence of bone ingrowth into the cambium surface of the periosteum could be found at 4 weeks. Progressive collagen fiber-bone anchorage, maturation and organization on the interface fibrous layer developed at 8 and 12 weeks. For biomechanical tests, the tibia with affixed tendon was posted in a MTS machine for measurement of maximal pull-out load (interface strength). At 4 and 8 weeks the majority of the periosteum-treated limbs failed by rupture in the midsubstance of the muscle or at the muscle-tendon junction, while the control limbs still failed by tendon pullout from the bone tunnel. Gross and histologic analysis of several specimens after pull-out in the periosteum-enveloping tendon demonstrated that failure occurred at the junction of the tendon and the fibrous interface tissue. An interface tissue layer remained along the walls of the bone tunnel after tendon pullout. The periosteum-treated limb had higher interface strength than the paired control limb at 4 and 8 week time points. There was a significant increase in the interface strength between the periosteum and control group at 12 weeks. There was a significant increase in the interface strength between the 2- and 4-week specimens and between the 4- and 8-week specimens. The periosteum-treated limb had a higher interface strength-to-length ratio than the control limb at each time point, while there were no significant differences. There was a significant increase in the interface strength-to-length ratio between the 4 and 8 week specimens and between the 8- and 12-week specimens.

2. BMP-2-fibracol for tendon-bone healing in ACL reconstruction

Tendon-bone healing within the bone tunnel is a major concern when using tendon graft for ligament reconstruction. BMP-2 (Bone

morphogenetic proteins) has been regarded to have positive effects on ligament fibroblast proliferation that resulting in healing. 24 adult New Zealand white rabbits were used. ACL reconstruction with long digitorum extensor tendon was performed In both limb. A 1 cm x 1 cm of fibracol membrane with BMP-2 was sutured on the both side of the tendon graft portion. The contralateral limb with only fibracol membrane on the tendon was used as a control. The rabbits were sacrificed at 4, 8, 12 weeks after operation. Histological analyses for the tendon-bone interface and pull-out strength tests for the interface strength were examined. The tendon-bone interface in the BMP-2-fibracol membrane group showed interface fibrous layer with progressive organization during the tendon-bone healing process. Evidence of bone-ingrowth into the interface layer could be found at 4 weeks. More extensive bone formation around the tendon with closer apposition of new bone to the tendon in the BMP-2 group could be found. Progressive growth of tendon fiber into bone tissue over the interface developed. Maximal pull-out load of the BMP-2 group were statistical significant better than the control group at 8 and 12 weeks. Positive effects were demonstrated in our study, where application of BMP-2 significantly increased the ultimate pull-out load compared with the control group. BMP-2 could enhance the integration between tendon and bone within a bone tunnel.

3. Oteoprogenitor cells-hydrogel for tendon-bone healing in ACL reconstruction

Fixation and incorporation of a tendon graft within the bone tunnel is a major concern when performing ligament reconstruction. Bone morphogenic signaling protein can augment tendon-bone healing. The purpose of this study is to develop a bioscaffold that both support biospecific cell adhesion via tethered signaling ligands and encourage robust extracellular matrix synthesis for tendon graft-to-bone healing in a rabbit model. Hydrogel was prepared from 10% of poly (ethylene glycol) diacrylate (PEGDA) dissolved in PBS containing 0.05% of the photoinitiator (Irgacure 2950). Bone morphogenic protein-2 (BMP-2) tethered hyaluronan (HA) conjugate was blending to the hydrogel. The long digital extensor tendon was cut on the lateral femoral condyle and translated through a 2.0-mm diameter tunnel created in the proximal tibial metaphysis. Rabbit osteoprogenitor cells (OPCs) isolated from

periosteum were resuspendended in co-gel solutions at a concentration of 20 million/ml, then were injected in the bone tunnel. The contralateral limb received the same procedures without cells injection. Ultraviolet irradiation (365 nm) was applied for 60 sec to photopolymerize the injections. The rabbits were allowed to recover, and then sacrificed at 2, 4, and 8 weeks postoperatively. The morphological characteristics of the healing tendon-to-bone interface were evaluated by histological and immunohistochemical methods. Serial histological and immunohistochemical analysis of the tendon-bone interface showed that an interface fibrous layer formed by photopolymerized hydrogels encapsulated OPCs. At 4 weeks, the new cancellous bone lined the bone tunnel, which became interdigitated with interfacial fibrous tissue. At 8 weeks, there was progressive mineralization and maturation of de novo bone ingrowth the interfacial fibrous layer. The specimens with OPCs exhibited more perpendicular collagen fiber formation and increased proliferation of cartiage-like cells, which was indicated by positive collagen type II immuno-staining of the tendon-bone interface. PEG-based hydrogel provided a suitable microenvironment for the osteoblastic growth and differentiation. Our results indicate that BMP-2 can be tethered to HA, and retain its activity to increase osteogenesis. We have reported in vivo results of successful tendon graft-to-bone healing using periosteum. Photoencapsulation of a large number of OPCs to the bone tunnel have shown to improve the insertion healing of tendon to bone in a rabbit model through the neoformation of fibrocartilagenous attachment and bone. The current approach is being refined for tissue-engineered therapeutic applications.

Enhancement of tendon to bone healing in ACL reconstruction – clinical application

1. Periosteum-enveloping tendon graft for tendon-bone healing in ACL reconstruction

We applied it for ACL reconstruction when using hamstring tendon graft. We believe that periosteum can positively affect graft healing and thus allows for more aggressive postoperative rehabilitation. Periosteum is easy to harvest from proximal tibia that is routine incision for hamstring tendons harvesting. Periosteum can be expected to seal off the intraarticular tunnel opening in a very early period due to the potent

integration between cambium layer and cancellous bone. This will avoid synovial fluid influx between the tendon and the bone Periosteum may be even more effective in situations in which healing may be impaired, such as those in which there is excessive graft-tunnel motion, or bone-tunnel widening. The graft is composed of double loops of semitendinosus and gracilis tendon, measuring 10 cm in length. A 3 cm x 3 cm periosteum flap was harvested from anterior tibial cortex through the tibial incision. The periosteum flap was then divided to create two 3 cm x 1.5 cm flaps. The periosteum was wrapped with the cambium layer placing outside to face the tunnel wall and then sutured on the tendon at both sides where the tendon graft approaches the tunnel opening. Clinical assessments included the Lysholm knee scores, International Knee Documentation Committee (IKDC) scores, KT-1000 instrumented testing, thigh muscle assessment, and radiographic evaluation. The median Lysholm knee score was 59(40~70) and 94(60~100) points ($P<0.01$) before and after surgery. After reconstruction, 81% of patients were able to return to moderate or strenuous activity. 6% patients were found to exhibit grade 2 or more ligament laxity. Complete range of motion could be achieved in 86% of patients. 5% had positive pivot shift. Finally, 92% of patients were assessed as normal or nearly normal rating by IKDC guideline. Bone tunnels enlargement of more than 1 mm was identified in 5% of femoral tunnels and 6% of tibial tunnels. Satisfactory result can be achieved with the periosteum-enveloping hamstring tendon graft in ACL reconstruction. Periosteum can be easily harvested at the proximal tibia from a routine incision for hamstring tendon harvesting. Besides the potential for improving tendon-bone healing, enveloped periosteum may help to seal the intra-articular tunnel opening in the early postoperative period, and thus avoid synovial fluid reflux into the tunnel. Bone tunnel enlargement could be reduced.

2. BMP-2-fibracol for tendon-bone healing in ACL reconstruction

For clinical application of growth factor on surgery, there are still some limitations. The limitations consist of the followings:

a. Cost of growth factors.
b. Short half-life time of growth factors.
c. Unreliable delivery or vehicle system for growth factors.
d. Need to develop a slow-release system to carry growth factors.

3. Oteoprogenitor cells-hydrogel for tendon-bone healing in ACL reconstruction

There are some problems for clinical application of progenitor stem cell to enhance tendon-bone healing in ACL reconstruction at present. The problems include the followings:

a. Need a pre-reconstruction surgery to harvest tissue (bone marrow or periosteum) for stem cell culture.
b. Arthroscopic techniques to apply the stem cells to tendon bone interface.
c. Need to develop an adequate delivery system for stem cells.
d. Consider the cost-effective issue.
e. Need to develop a easier and more realistic progenitor cells source such as from peripheral blood.