Reconstruction of Rabbit Anterior Cruciate Ligament Using the Autogenous Patellar Tendon

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INTRODUCTION

The anterior cruciate ligament (ACL) deficient knee is usually reconstructed by using prosthetic augmentation, autogenous graft or allograft under arthroscopy.

Many kinds of artificial ligaments are available, which include Kennedy-LAD (13, 10), Leeds-Keio (5), GORE-TEX (2) and Dacron Ligaments (12). However, they are susceptible to degradation, as a result of it, fiber failure and the generation of ligament wear particles occur with time. Knee's reaction to wear debris results in severe synovitis, response to foreign body and caseous necrosis leading to the modification or abandonment of implant devices.

Allografts take a longer time than do autogenous grafts for revascularization and replacement by host cells and collagen fibers because of prolonged protection of the knee. In addition, little is known about allografts in the aspects of immunogenecity, preservation and secondary sterilization, remodeling and its effects on mechanical properties, the potential of disease transmission, and most importantly, the procurement of harvest bone. Autogenous grafts have no such problems inherent to allografts, they need to sacrifice soft tissue or bone. Therefore, most surgeons prefer to use autogenous graft, particularly the central one-third of the patellar ligament, for intraarticular reconstruction of the anterior cruciate ligament.

However, the maturation process of autografts still remains to be clarified. This study was designed to grossly and histo-logically examine changes with time in the patellar tendon used as autogenous graft for ACL reconstruction.

MATERIALS AND METHODS

Forty-three Japanese white rabbits, weighing a mean of 3.0 kg were anesthetized with an intramuscular injection of ketamine hydrochloride 1-2 ml (Ketalar50, Sankyo Pharmaceutical Co., Ltd.) and an intravenous injection of pentobarbital sodium 1-2 ml (Nembutal, Dainippon Pharmaceutical Co., Ltd.). Each animal underwent a medial parapatellar incision on the skin to expose the patellar tendon and the intraarticular space. This tissue was stripped from its underlying tissue so as to comprise the medial third of the quadriceps-
patellar tendon and include a portion of the patellar bone without articular cartilage. The medial third of the patellar tendon was separated with a small fragment of the patellar bone and quadriceps tendon. But the tibial-side patellar tendon insertion was preserved without cutting (modified Jones (9) method). The original anterior cruciate ligament was then excised. A 3.2 mm diameter tunnel was drilled from the intercondylar space at the original anterior cruciate ligament area to the lateral condyle with the leg in 30° of flexion. The medial patellar tendon was passed through the intraarticular tunnel, pulled out and fixed to the lateral condyle using silk suture with adequate tension (Fig. 1). Postoperatively each rabbit was put in a cage (50 cm x 45 cm x 32 cm) without external fixation.

The animals were sacrificed from 2 to 48 weeks after reconstruction. Forty-three reconstructed knee joints were grossly and histologically examined: 3, 8, 8, 7, 5, 9, and 3 knees at 2, 4, 6, 12, 16, 24 and 48 weeks after operation, respectively. Specimens for histological examinations were prepared according to the standard procedures, i.e., by decalcifying the knees, sectioning them into 4-6 μm cuts and staining them with hematoxylin–eosin, azan-mallory and alcian-blue. The specimens were examined under non-polarized light microscopy.

RESULTS

Physical and gross examinations

Physical examinations at sacrifice revealed some degree of anterior-posterior laxity in all of the 43 knees.

Gross inspections throughout the study revealed that the surfaces of the patellar tendon autografts were already covered with a thick synovial sheath. The grafts showed a gross atrophic change in 1 knee at 4 weeks, in 2 knees each at 6 and 16 weeks, and in 2 knees at 24 weeks. One graft in the 2 atrophic grafts at 24 weeks showed a severe atrophic change (Fig. 2).

Throughout the study the grafts were in lusterless whitish color, however no signs of necrosis, edema, tearing, disappearance or slippage were apparent. Intraarticular fluid increased moderately and its viscosity decreased. There was a hypertrophic change in the synovial tissue and infrapatellar fat pad. The hypertrophic synovial tissue was in the shape of a fan in the graft insertion sites around the femur and tibia, especially around the latter. The synovial tissue in the mid-zone of the grafts did not show a hypertrophic change, although a few longitudinal bundles of the tissue were seen along the grafts running from the tibia to the femur. Observations of the cross section showed that the grafts tended to

Fig. 1 A method to reconstruct the ACL deficient knee with the medial third of the patellar tendon.

Fig. 2 The patellar tendon graft showed a severe atrophic change (arrow) at 24 weeks.
slightly increase in size with roundness until 8 weeks. At the same time the autograft surfaces were covered with a thick synovial sheath. At 12 weeks postoperatively, the cross section of the grafts showed a slight decrease in size.

HISTOLOGY

At 2 and 4 weeks postoperatively, there were bundles of fibroblastic connective tissue with hypercellularity beneath a layer of synovial tissue (the peripheral portions) that surrounded the grafts. Cellular invasion appeared to occur markedly from the surrounding of the grafts. Fibroblasts were seen scattered with microscopic vessels throughout the length of the grafts. The core of the grafts began to show degeneration, a-or hypocellularity, and collagen fragmentation (Fig. 3).

Although it was difficult to differentiate bundles of the fibroblastic connective tissue from the immature cellular collagenous tissue, the collagen fibrils and cells with spindle-shaped nuclei were more stress-oriented longitudinally at 6 and 8 weeks postoperatively than at 4 weeks. Microscopic vessels were still found. Inflammatory cells were present on the surface of the grafts until 6 weeks.

At 12 and 16 weeks postoperatively the grafts were still hypercellular, although mature collagen bundles were found with spindle-shaped nuclei. On the whole, the vascularity in collagen bundles and the shape of nuclei were similar to those in the normal ligament. The sections of the grafts did not reveal degeneration (Fig. 4).

At 24 and 48 weeks postoperatively, the grafts appeared to be more completely remodeled with wider bundles and less vascularity. No microscopic degeneration were found in residual areas. The grafts were not hypercellular and individual fibroblasts had resumed their narrow-spindle shape. Examinations under non-polarized light at 24 and 48 weeks suggested that sufficient remodeling occurring to produce mature collagen bundles in alignment (Fig. 5).

The intraosseous attachments of the autografts.

The cross-sections of the femoral tunnel at 2 weeks showed a profuse fibroblastic response and fibrocartilage about the periphery of the tunnel. The interface between the autograft and the sides of tunnel appeared to be filled with immature tissue until 4 weeks and with mature tissue from 6 weeks on. The longitudinal sections of the femoral tunnel at 4 weeks showed a profuse fibroblastic and osteoblastic response as well as mineralized fibrocartilage about the periphery of the tunnel. In some areas of the attachment the interface between the ligament substitute and the sides of the tunnel appeared to be filled with fibrous tissue.

Fig. 3 Cellular invasion appeared to occur markedly from the surrounding of the graft. The core of the graft showed degeneration, acellularity and collagen fragmentation at 4 weeks. (HE, x20)
Fig. 4 The vascularity in collagen bundles and the shape of nuclei at 12 weeks were similar to those in the normal ligament. (HE, x50)

Fig. 5 Mature collagen bundles were produced in alignment at 24 weeks. (HE, x50)

Fig. 6 The insertion site of the patellar tendon at the femoral tunnel at 12 weeks was similar to attachments of the normal tendon or ligament to bone. (HE x20)
tunnel at 8 weeks was markedly filled with oriented osteoblasts. At 12 weeks postoperatively, there were zones similar to those described for attachments of the normal tendon or ligament to bone (Fig. 6). At 24 weeks these zones were composed of ligament, fibrocartilage, mineralized fibrocartilage, and bone.

DISCUSSION

Even though reconstructed ligaments in the intraarticular space are of fascia, tendon of ligament origin, they are remodeled with time following histologically degenerative processes. Patellar tendon autograft for the ACL deficient knee is frequently used because of its good mechanical properties. Butler et al. (4) found that the strength of one third of the patellar tendon was almost twice (191 percent) that of the normal ACL in humans, whereas the semitendinosus was only 75 percent of the normal ACL in strength. Another benefit of using the patellar tendon is that it permits bone-to-bone union at the insertion sites. This does not deprive the knee of a significant stabilizer, as is the case when the iliotibial tract or semitendinosus tendon is used for reconstruction of the ACL.

The transplanted tendon is essentially a free graft and must therefore be revascularized entirely (11). However, the graft used to reconstruct the ACL deficient knee as in this study does not seem to receive any nutrients from osseous attachment, although it is an isolated patellar tendon graft that is left attached to the tibia. According to literature, the graft initially depends upon the nutrients diffusing from the adjacent synovial tissue and synovial fluid (7). There is additional evidence that autografts can survive on synovial nutrition without vascularization. Amiel et al. (1) used auto-radiographic techniques in a study of immature rabbits and found that tritiated proline was taken up by knee ligaments from the synovial fluid. Fulkerson et al. (6) and Bruce D. et al. (3) found that the autograft can survive from synovial nutrition alone, and that it is possible that the graft can remain viable until early vasclarization occurs.

Imai (14), Grillo (8), and Ross (15) found that the fibroblasts originate from the local connective tissue and synovium, invading into the intercollagenous bundles to remodel the grafts. Kondou (11) supported this view, emphasizing the necessity of the grafts to be covered with synovium.

Patellar tendon autografts are frequently used as free bone-tendon-bone grafts in human for ACL reconstructions. Many surgeons allow patients with an ACL reconstructed knee to return to their daily life within 6 months after surgery. However, the graft maturation deserves much more consideration to determine appropriate postoperative management. With this in mind, we designed to study the natural response of the patellar tendon autograft in rabbits. Although the histology of rabbit patellar tendon autografts for ACL reconstruction differs greatly from human studies, the reconstructed grafts appeared to be completely remodeled by 24 weeks postoperatively. It should be noted, however, that the relaxation of the autograft occurs during those 24 weeks probably due to incomplete response to tension or stretch by inadequate rehabilitation and minor trauma.

As stated above, the transplanted autogenous patellar tendon graft undergoes a series of processes of necrosis, revascularization and proliferation until remodeling. This indicates that adequate rehabilitation has to be carefully performed throughout the period of the ligamentization.

REFERENCES

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