Attachment of autogenous tendon graft to cortical bone is better than to cancellous bone

A mechanical and histological study of MCL reconstruction in rabbits

Yoshinori Soda[1], Yoshio Sumen[2], Yuji Murakami[3], Yoshikazu Ikuta[4] and Mitsuo Ochi[3]

Departments of Orthopedic Surgery, [1]Hiroshima City Hospital, 7-33 Motomachi, Naka-ku, Hiroshima 730-8518, [2]JA Onomichi General Hospital, Hiroshima, [3]Hiroshima University School of Medicine, Hiroshima, [4]Hiroshima JR Hospital, Hiroshima, Japan.
Correspondence: ysoda35@ybb.ne.jp
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ABSTRACT **We analyzed the mechanical and histological variables after the attachment of an autogenous tendon graft to cortical or cancellous bone. We reconstructed the medial collateral ligament of the knee in 33 Japanese white rabbits, using a bone socket procedure. The floor of the bone socket was cortical bone in group A and cancellous bone in group B.**

Mechanically, the pull-out test showed a tendency towards an increase in maximum failure load, with 10.9 N, 35 N and 37 N in group A, and 11 N, 18 N and 36 N in group B at 2, 4 (statistically significant difference) and 8 weeks after surgery, respectively. Histologically, the attachments were immature at 2 weeks. At 4 weeks, granulations had matured and Sharpey's fiber-like structures were seen. These fibers were more abundant in group A than in group B. At 8 weeks, the attachments in both groups were rather like the normal 4-zone structure. With time, tendon attachments matured in both groups.

Our study showed that reattachment of tendons to cortical bone may be bettter than to cancellous bone.

One of the keys to successful tendon grafts for knee ligament reconstruction, using the bone tunnel procedure, is early and firm attachment of the graft to bone. It has been traditionally taught that tendon healing to bone is enhanced by attaching the tendon to a cancellous trough or through a hole in the bone (Kernwein 1942, Whiston and Walmsley 1960, Forward and Cowan 1963, Ward et al. 1988). Repair by direct attachment to cortical bone has been regarded as inferior, since it has been assumed that an intervening layer of mechanically inferior connective tissue would develop and impair healing (Jones et al. 1987). Tendon attachment to bone in cases where cancellous bone surrounds the graft, has been considered better than in those with surrounding cortical bone, because mesenchymal cells from bone marrow will presumably invade the tissue. However, the insertion of a normal ligament is into cortical bone (Cooper and Misol 1970), therefore cortical bone may be the most suitable for tendon reattachment.

We evaluated the mechanical and histological variables in cortical and cancellous bone after medial collateral ligament reconstruction, using bone sockets in a rabbit model.

Animals and methods

Surgical procedure

We used 33 Japanese white rabbits, each weighing an average of 3.3 (3.0–3.5) kg. The animal experiments were done in accord with the Rules and Regulations of Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.

In each animal, bilateral medial collateral ligament (MCL) reconstructions were performed as follows (Figure 1). Each animal was anesthetized with an intravenous injection of pentobarbital

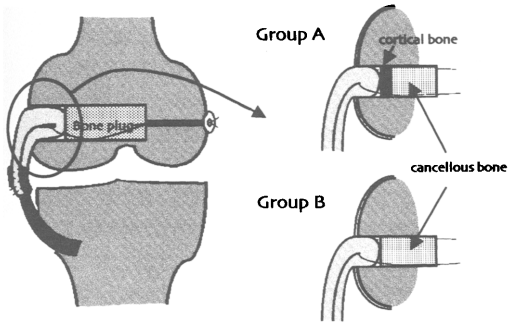

Figure 1. MCL reconstruction model using a bone socket. The floor of the bone socket is cortical bone in group A, and cancellous bone in group B.

sodium, 35 mg/kg. An arthrotomy was performed via a medial parapatellar incision. The MCL was detached from the femoral insertion by sharp dissection, and reflected. The periosteum was removed and cortical bone scraped with a scalpel at the medial femoral condyle to create a bone socket. A 10 mm deep bone socket was made by removing a 10 mm long bone plug with a trephine of 3.5 mm in diameter at the medial femoral condyle where the MCL had been cut. The bone plug was trimmed by 3 mm in length to a final length of 7 mm. In group A (right knee), the bone plug was trimmed from the cancellous bone end, creating a plug with one end of cortical bone and the other end of cancellous bone. In group B (left knee), the bone plug was trimmed from the cortical bone side, creating a bone plug of cancellous bone alone. The graft for MCL reconstruction was the free semitendinosus tendon, harvested with the adductor muscle retracted anteriorly. The trimmed bone plug was reinserted into the bone socket with a double loop of semitendinosus tendon, which was attached to the untrimmed side of the bone plug (i.e., cortical bone side in group A, cancellous bone side in group B). The bone plug was introduced, using a 3-0 nylon suture, through a hole made with an injection needle (1.20 mm diameter) from the end of the bone socket to the lateral aspect of the lateral femoral condyle. A nylon suture was tightly tied to a nut on the lateral femoral condyle to maintain the position of the bone plug and the graft. The graft was inserted to a depth of 3 mm into the bone socket. The graft outside the bone socket was tied with MCL detached from the medial femoral condyle, using 8–10 4-0 nylon sutures, under moderate tension maintained throughout the full range of motion of the knee. The knee was stable during varus and valgus stress tests. The wound was washed out and closed in layers. After the surgery, the rabbits were returned to their cages and allowed unlimited activity.

Tissue preparation

At 2, 4 and 8 weeks following surgery, 8, 13 and 12 rabbits were killed with an overdose (100 mg/kg body weight) of pentobarbital sodium. After cutting the reconstructed MCL at the tibial insertion, the femoral condyle was harvested and trimmed by removing other soft tissues (ligaments, tendons and capsule) and leaving only the MCL. Thus, the graft and femoral condyle complex was harvested as a specimen.

Mechanical testing

Maximum failure load was measured at 2, 4 and 8 weeks after surgery. 6, 8 and 8 rabbits were used for each period, respectively. The graft and femoral condyle complexes were kept in 0.9% saline and tested within 3 hours of excision. The nut on the lateral femoral condyle was removed after cutting the nylon suture and the femoral condyle was fixed with Poly Maleinate Glass Ionomer Cement (Ketac-Cem radio-opaque, ESPE, Germany) in a specially-designed chrome case. We used a Shimadzu Autograph AGS 1000A (Shimadzu, Tokyo, Japan) for mechanical testing. The graft was clamped at 10 mm from the entrance of the bone socket with a load cell. The femoral condyle fixed with cement in a case was mounted facing the graft. The graft was pulled superiorly at a rate of 50 mm per minute, in line with the long axis of the bone socket until failure. Maximum failure load was measured without preconditioning of the tendon.

Histological examination

The histological examinations were done at 2, 4 and 8 weeks after surgery. 2, 5 and 4 rabbits were used for each period, respectively. Specimens, drop-fixed in 4% paraformaldehyde for 72 hours and decalcified in ethylenediaminetetraacetic acid in phosphate buffered saline for 3 days, were dehydrated in graded ethanol and then embedded

in paraffin. The tissue samples were sectioned longitudinally 10-mm thick along the axis of the bone socket, mounted on microscope slides, and were stained with hematoxylin and eosin.

We examined the tendon attachment to the bone in the floor of the bone socket with light and polarized light microscopy.

Statistics

The statistical analysis at each period was done, with an unpaired t-test to determine the significance of the differences between groups A and B as regards the mean values of maximum failure load. A one-way factorial analysis of variance (ANOVA) test was used to compare the data at different times within each group.

The threshold for statistical significance was set at $p < 0.05$.

Results

Mechanical results (Table)

Two types of failure were evaluated. The first, pull-out failure, occurred when the grafted tendon was entirely pulled out of the bone socket. The second, tendon substance failure, occurred when any tear was seen in the grafted tendon substance itself.

In both groups all failures of the graft-femur complex were of the pull-out failure type at 2 and 4 weeks after surgery and 8 weeks after surgery, 3 of 8 cases were of the pull-out failure type and the other 5 were of the tendon substance failure type.

We found significant differences in mean values between both groups at 4 weeks after surgery ($p = 0.006$), but not at 2 and 8 weeks after surgery.

The differences in the mean value of the maximum failure load between weeks 2 and 4 in group A ($p = 0.007$), and between weeks 4 and 8 in group B ($p = 0.03$) were also significant.

Maximum failure load. Mean (SD)

Weeks after surgery	Group A	Group B
2	11 (6.0) [a]	11 (4.6)
4	35 (12) [a, b]	18 (10) [b, c]
8	37 (17)	36 (20) [c]

[a] $P = 0.007$
[b] $P = 0.006$
[c] $P = 0.03$

Histological results

At 2 weeks after surgery. In both groups, we found proliferating fibrous tissue, fibroblasts and newly formed vessels (granulations) in the void spaces of trabeculae and giant cells with multiple nuclei in some areas. Fibrous tissue was aligned along the bone wall. At the entrance of the bone socket, distinctive inflammatory cells and disorganized fibrous tissues were seen. In the adjacent bone, new bone had formed, which contained a mixture of osteoclasts and osteoblasts. The tendons attachment to bone was histologically immature. Polarized microscopy showed poor organization of tissues and little, if any, collagen continuity between the graft and adjacent bone.

At 4 weeks after surgery. The width of fibrous tissues decreased in the bone marrow cavity. Fibroblasts showed a more parallel arrangement. Sharpey's fiber-like structures were seen extending vertically from the floor of the bone socket to granulations or directly to the tendon, while those from the bone to the graft had become, aligned with the grafted tendon in a part of the bone wall. Near the entrance, the gap between the graft and bone was filled with which were poorly organized granulations, and showed little collagenous continuity. The fibrin was separated in some places. Using light and polarized light microscopy, we found that lamellar bone was more abundant in the floor of the bone socket and Sharpey's fiber-like structures were much commoner in group A than in group B (Figure 2).

At 8 weeks after surgery. The fibrous tissues had become more mature, and tendon attachments to the bone resembled the normal 3 or 4 zone structures in parts of the bone socket. The attachments were far more mature in the floor of the bone socket than along the bone wall in group A. At the entrance, the graft was connected with fibrous tissues outside the bone socket rather than with bone. Histologically, tendon attachments had matured in both groups during this period.

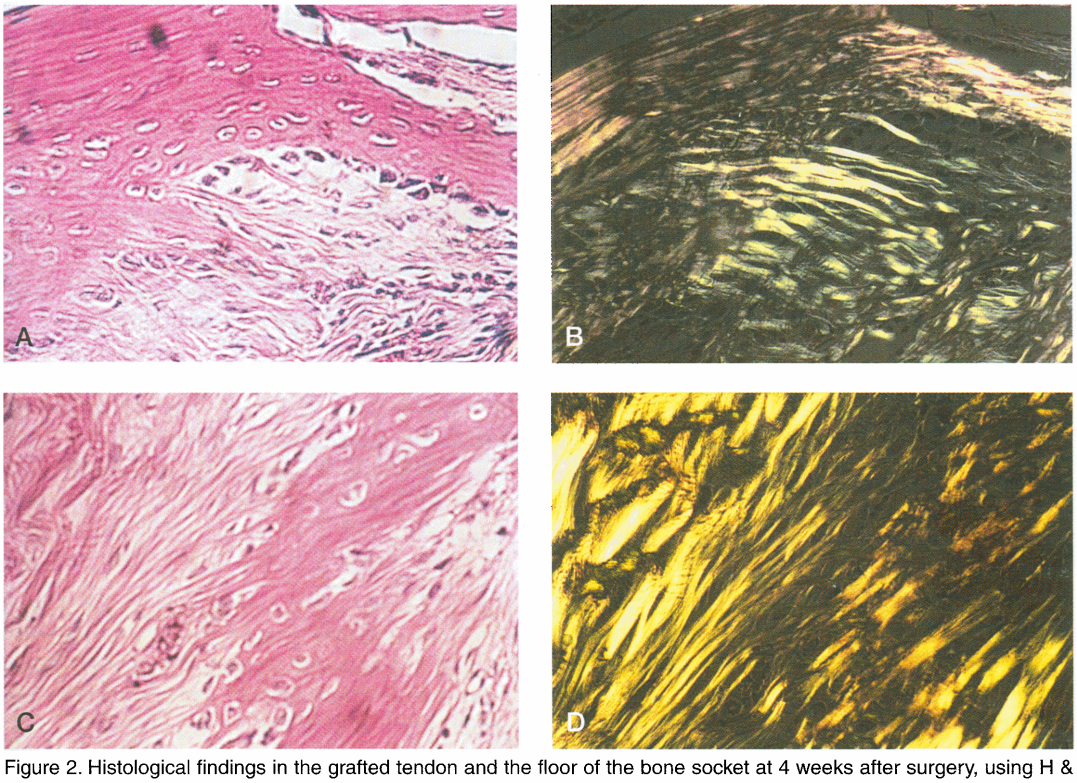

Figure 2. Histological findings in the grafted tendon and the floor of the bone socket at 4 weeks after surgery, using H & E stain (original magnification, ×200). In group A, in section (A), shows some Sharpey's fiber-like structures from bone to tendon, using light microscopy. With polarized microscopy (B), in the same field as (A), fibrous continuity is visible between graft and bone. In group B, the finding with light microscopy (C) and polarized light microscopy (D) were largely the same as in A and B, respectively. However, more lamellar bone and many more Sharpey's fiber-like structures were seen in group A than in group B.

Discussion

Although the histological findings were similar on each examination, especially at the entrance and the wall of the bone socket, the mean values of maximum failure loads at 4 weeks after surgery were higher in group A (cortical bone) than in group B. In our view, the attachment of tendon to cortical bone containing more lamellar bone with an abundance of Sharpey's fiber-like structures was stronger than that to cancellous bone.

At 8 weeks after surgery, we found no differences between the groups on mechanical testing. Moreover, the standard deviation of maximum failure load at 8 weeks was greater than that at 2 and 4 weeks. This may have been due to the 2 types of failure, pull-out failure and tendon substance failure; therefore the mean values of maximum failure load at 8 weeks did not explain the strength of attachment between the grafted tendon and bone, which would suggest that the weakest points changed from the new bone-tendon interface to the graft itself during this period.

One of the keys to successful tendon grafts for knee ligament reconstruction using the bone tunnel procedure is early and firm attachment of the graft to bone. The 2 weakest points in reconstructive surgery for knee ligaments using autogenous tendon grafts through bone tunnels are the tendon-bone interface, and graft strength with time. These factors affect postoperative rehabilitation at an early phase. Although follow-ups of graft survival have been done in the past, the literature regarding tendon-bone attachment remains scanty. Since Gallie and Mesurier's study (1922), the reports by Kernwein (1942), Whiston and Walmsley (1960) and Forward and Cowan (1963) have been published. In recent years, the number of studies has

gradually increased along with the remarkable progress in knee ligament surgery. Rodeo et al. (1993), Grana et al. (1994) and Blickenstaff et al. (1997) recently reported that the attachment of tendon to bone formed a 4-zone structure in normal ligamentous insertions (Cooper and Misol 1970), and that maturation and organization of granulations at the tendon-bone interface occurred along the bone tunnel wall of newly formed bone. It was also shown that Sharpey's fiber-like structures anchored tendons or granulations to bone, rather than that bone grew into tendon, or that the tendon itself because ossified.

Sharpey's fibers, as described by Sharpey and Ellis (1856), are perforating fibers that project "like nails driven perpendicularly or slanting through a board" from the tendon into cortical bone. They are thought to be the primary fibrous tissues anchoring tendon to bone. Our findings indicate that these fibers, projecting from lamellar bone into tendinous or granulation tissues around the tendon, play an important role in the attachment of graft to bone.

It is thought that cancellous bone around the graft, mesenchymal cells from bone marrow will invade the tissue. Most authors have therefore assumed that attachments of autogenous tendon graft to cancellous bone are better than to cortical bone, in accord with the view of Jones et al. (1987). However, no studies have been done to test this assumption. When one considers that the normal insertion of ligament is into cortical bone, and that some authors have noted a marked maturation of reattachments at the entrance of bone tunnels after ligament reconstruction (Ward et al. 1988, Hausman et al. 1989, Rodeo et al. 1993), it becomes uncertain whether cortical or cancellous bone is more suitable for tendon reattachment.

So far as we know, only two authors (St Pierre et al. 1995, Shaieb et al. 2000) have studied this problem and both found that mechanical and histological testing gave similar results. However, in both studies, the tendons were fixed directly to the cortical bone, but in the cancellous bone, the grafts were inserted into a bone trough using the pull-out method. Although the bony quality around tendons differed between the groups, adjacent tissues may have affected the attachment of grafts to bone under such different conditions. These authors, therefore, may have arrived at incorrect conclusions.

Our conclusion is that the tendon attachment to cortical bone seems to be better than that to cancellous bone alone.

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