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A comparative study of the healing of tendon autograft and tendon-bone autograft using patellar tendon in rabbits

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Abstract In order to compare the healing of tendon to bone and the healing of bone to bone in a rabbit model, the lateral 4 mm of patellar tendons were detached from their insertion into the tibia either subperiosteally (group I) or with a bone block (group II) and implanted into drill holes in the proximal articular surface of the tibia. The histological and biomechanical features of the graft incorporation were observed at 2, 4, 8 and 12 weeks. Histological patterns similar to normal tendon-bone attachment were seen at the tendon-bone interface in group I by 12 weeks, while direct bony union was seen in group II by 8 weeks. The maximum tensile load and stiffness were significantly greater in group II at 4 and 8 weeks while the difference between the two groups was not significant at 2 and 12 weeks. These findings show that more rapid incorporation of the graft occurs in group II although no significant difference in biomechanical parameters was noted once healing was complete.

Résumé Pour comparer la cicatrisation de tendon à os et la cicatrisation d'os à os chez le lapin, on a séparé une bande latérale de 4 mm du tendon rotulien soit en sous-périosté (groupe I), soit avec une pastille osseuse (groupe II). Et puis, on l'a implantée dans le trou foré dans la surface articulaire proximale du tibia. Enfin, on a observé l'aspect histologique et biomécanique de l'incorporation de la greffe à la 2ème, 4ème, 8ème et 12ème semaine. On trouve un aspect histologique habituel de la jonction os-tendon normal dans le groupe I à 12 semaines tandis que 1'on peut voir 1'union osseuse directe dans le groupe II à 8 semaines. La charge admissible maximale et la résistance sont plus élevées dans le groupe II à la 4ème et 8ème semaine mais non aux semaines 2 et 12. Mais la différence entre deux groupes n'a pas été significative à 4ième et à 12ième semaine. Cela démontre que 1'incorporation de la greffe dans le groupe II se produit plus rapidement. Mais il n'y a pas de différence significative dans les paramètres biomécaniques après cicatrisation.

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Introduction

Understanding the mechanism of graft incorporation into bone is essential for successful reconstruction of knee ligaments using autograft structures. Among the techniques available for the reconstruction of knee ligaments, bone-patellar tendon-bone has been favoured by many surgeons as graft incorporation is achieved through bone-to-bone healing [1, 4, 5]. However, a bone-tendon-bone structure is only provided by the bone-patellar tendon-bone technique. The increasing popularity of using tendons such as semitendinosus and gracilis has made it necessary to understand the incorporation process of the tendon graft at the insertion site, as post-operative rehabilitation depends on the histological restoration of a normal tendon-bone interface.

The purpose of this study was to compare biological properties of tendon-to-bone healing and bone-to-bone healing using autograft structures in an animal model.

Materials and methods

Skeletally mature New Zealand white rabbits weighing 3.4–3.8 kg were used. We divided 80 rabbits into two groups: group I, 40 rabbits which underwent transplantation of patella – patellar tendon autograft into the proximal tibia; and group II, 40 rabbits which underwent transplantation of patella – patellar tendon-bone autograft into the tibia. The animals were anaesthetised with intramuscular ketamine (40 mg/kg) and xylazine (10 mg/kg). A longitudinal lateral parapatellar incision was made on the left knee and the patellar tendon exposed. The lateral 4 mm of tendon were detached from the tibial tuberosity by two methods: subperiosteal

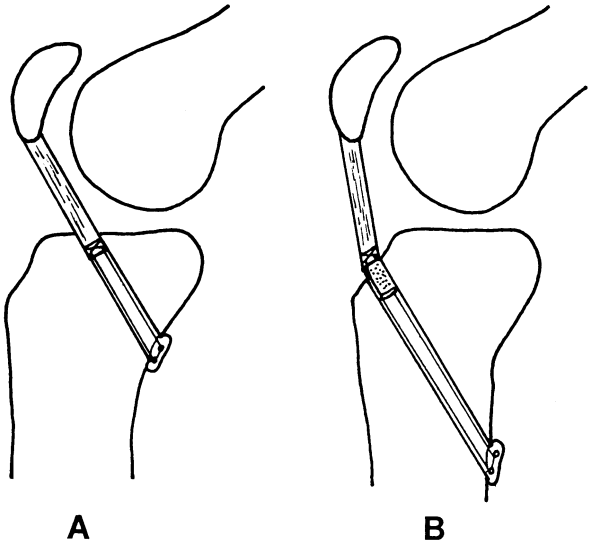

Fig. 1A,B Schematic drawing of the procedures in this study. The lateral 4 mm of patellar tendons which were detached from tibial insertion sites either subperiosteally (**A** group I) or with a bone block (**B** group II) were implanted into drill-holes in the proximal articular surface of the tibiae

dissection of tendon only in group I, and harvesting with a bone block 7 mm long, 3 mm thick and 3 mm wide in group II. A 3.5-mm hole was drilled into the articular surface of the proximal tibia directed distally and medially after dislocation of the patella. The lateral portion of the patellar tendon was implanted into this hole in group I while patellar tendon with the bone block was implanted in group II. In order to fix the tendon graft into the tibia, a Bunnell suture using 4-0 monofilament of nylon was inserted within the distal 4 mm of the tendon and withdrawn through the tibial tunnel and secured over a button. The tension of the transferred tendon was adjusted such as to make it straight without displacing the patella inferiorly with the knee extended. The tibial drill holes in group II were made slightly anterior and inferior to that of group I in order to equalise the length of the intraosseous portion of the autografts (Fig. 1). The length of autograft within the tibial tunnel was approximately 5 mm. After surgery all the animals were allowed unrestricted activity within their cages (area 4,500 cm^2). For each group, ten rabbits were sacrificed at 2, 4, 8 and 12 weeks after implantation. We performed the histological studies on two rabbits and mechanical tests on eight rabbits at each time period to compare the groups.

Histological study. Soft tissues were removed leaving only the tibia and transplanted patellar ligament, and the specimens were fixed in 10% buffered formalin, decalcified in 5% nitric acid and embedded in paraffin blocks. Sections, 5 μm thick, were made through the osseous tunnel and stained with H&E and Masson's trichrome. They were examined under light microscopy.

Biomechanical study. Eight limbs for each time period and each group were tested. The femur and all soft tissues were carefully removed leaving the patella, transplanted patellar ligament and tibia. The suture material used to fix the graft was removed. The tibia was divided 10 cm below the joint line and embedded in methyl methacrylate, so that it could be fixed in a specially designed clamp, and the patella was also clamped. A tension-failure test was carried out at room temperature using a materials testing machine (Instron 8,500; Instron, Canton, Mass., USA). The structural unit was loaded along the long axis of the patellar ligament to failure at a rate of 50 mm/min. A load-deformation curve was recorded for each specimen, and the mode of failure recorded. The structural properties represented by maximum tensile load and stiffness were determined from the load-deformation curves. Stiffness was the slope of the curve in its linear region.

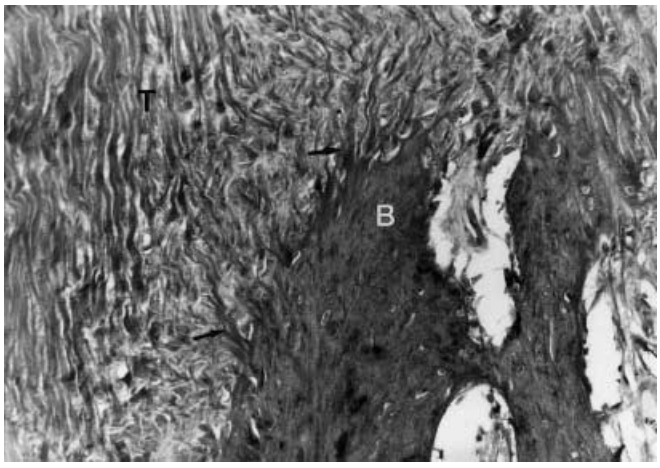

Fig. 2 Photomicrograph of group I specimen (tendon to bone) 8 weeks after implantation. The tendon (*T*) is continuous with the bone (*B*) through the perforating collagen fibres (*arrow*) which appear to be "Sharpey's fibres". Masson's trichrome, ×200

Statistics. Statistical analysis of the results was performed using the Wilcoxon rank sum test to determine if the structural properties of the specimens at each time period differed between the two groups. The level of significance was P=0.05.

Results

Histological findings

Group I (tendon-to-bone healing)

Specimens at 2 weeks showed vascular and cellular fibrous tissue between the tendon and bone. This tissue was poorly organised with little or no collagen continuity between the tendon and adjacent bone. At 4 weeks the collagen fibres of this tissue appeared to be continuous with those of the tendon, but rarely with the bone. At 8 weeks the interface was narrowed and more organised. Occasional perpendicular collagen fibres resembling "Sharpey's fibres" were seen crossing the tissue junction, and cartilaginous cells were also seen where the fibres were perforating the osteoid tissue (Fig. 2). At 12 weeks, there was increased maturation and organisation of the collagen fibre continuity; and a layered structure with four zones (tendon, fibrocartilage, mineralised fibrocartilage, bone), similar to the normal ligament to bone junction, was seen (Fig. 3).

Group II (bone-to-bone healing)

Specimens at 2 and 4 weeks showed that the inserted dense lamellar fragment was separated from the sur-

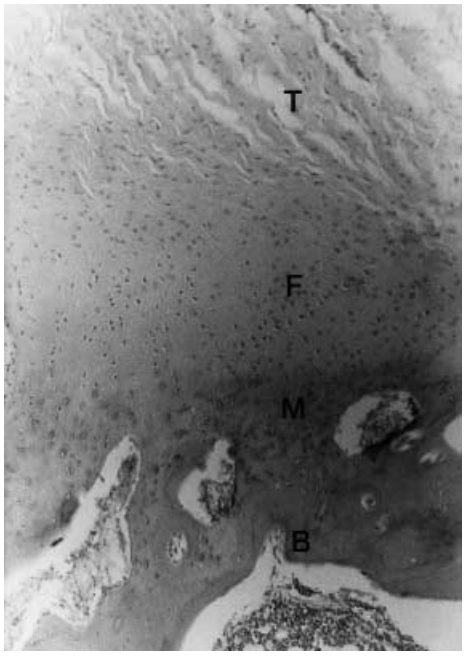

Fig. 3 Photomicrograph of group I specimen (tendon to bone) 12 weeks after implantation. Well-organized tendon-to-bone attachment is noted near the entrance to the bone tunnel. Four zones, tendon (*T*), fibrocartilage (*F*), mineralized fibrocartilage (*M*) and bone (*B*), can be distinguished like a normal tendon-to-bone insertion site. H&E, ×100

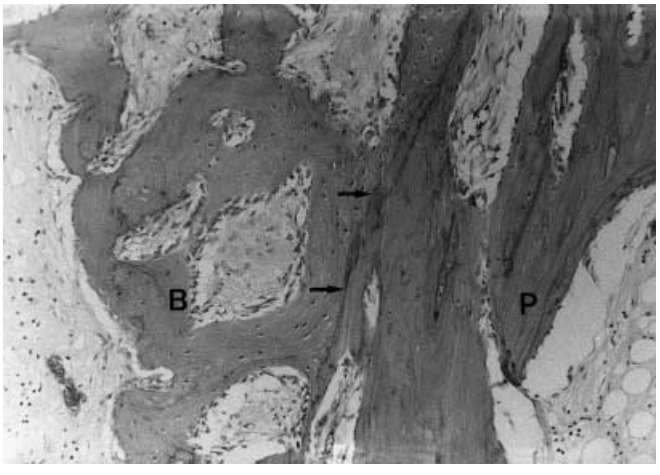

Fig. 4 Photomicrograph of group II specimen (bone to bone) 8 weeks after implantation. The junction of bone healing (*arrow*) between the inserted bone peg (*P*) and the less mature newly formed bone (*B*) lining the tunnel can be noted. H&E, ×100

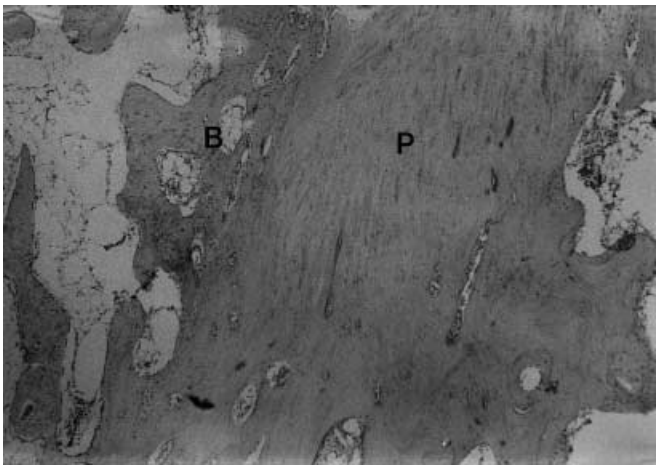

Fig. 5 Photomicrograph of group II specimen (bone to bone) 12 weeks after implantation shows increasing maturation of osseous healing between the bone peg (*P*) and the bone (*B*) lining the tunnel. H&E, ×40

Table 1 Maximum tensile load and stiffness of patella-patellar tendon – tibia complex of group I and group II. Means ± SD

Week	Maximum tensile load (N)	Stiffness (N/mm)
2 weeks		
Group I (*n*=8)	21.1±7.1	14.6±4.4
Group II (*n*=8)	22.9±9.5	17.1±7.0
4 weeks		
Group I (*n*=8)	62.8±13.1*	39.2±11.0*
Group II (*n*=8)	94.5±21.7*	62.0±10.8*
8 weeks		
Group I (*n*=8)	98.9±25.5*	44.6±9.2*
Group II (*n*=8)	142.6±33.8*	83.5±17.8*
12 weeks		
Group I (*n*=8)	200.4±43.5	77.6±20.0
Group II (*n*=8)	217.4±37.3	82.5±17.4
Normal control (*n*=10)**	267.9±29.3	136.9±29.7

* Value of group II is significantly higher than that of group I ($P<0.05$)
** The contralateral lateral 4 mm of patellar tendons with patella and tibial attachment were measured

rounding metaphyseal bone by fibrous tissue. No evidence of bone healing was found at 4 weeks. At 8 weeks, there was osseous incorporation between the two bony structures. The dense lamellar fragment of bone graft and newly formed osteoid with osteoblast rimming in the tunnel could be distinguished without intervening fibrous tissue. A basophilic line represented the interface between graft and host bone (Fig. 4). At 12 weeks, the area of osseous corporation had increased with the maturation of newly formed bone within the tunnel (Fig. 5).

Biomechanical testing

All 2-week and 4-week specimens of both groups failed by pulling the graft from the tunnel. In group I specimens tested at 8 weeks, five failed by pullout of the pa-

tellar tendon and three by tendon rupture near the entrance of the tunnel. All group II specimens at 8 weeks failed by rupture at the tendon-bone junction with or without a small avulsed fragment of bone, except one which failed by pullout of the entire graft. In all 12-week specimens of both groups there was failure of the tendon near the entrance of the tunnel.

The structural properties of both groups had increased from 2 to 12 weeks after implantation. At 2 weeks, the maximum tensile load and stiffness were not significantly different between the two groups ($P>0.04$). In contrast, group II showed significantly higher values than group I in terms of maximum tensile strength ($P=0.005$) and stiffness ($P=0.006$) at 4 and 8 weeks. At 12 weeks, the structural properties of group II maintained higher values than those of group I but without significant difference ($P>0.2$; Table 1).

Discussion

This study compared two different environments for the healing of graft to bone: tendon-to-bone healing and bone-to-bone healing. Our method has some limitations when applying the findings to cruciate ligament autograft reconstruction in that we did not perform anterior cruciate ligament reconstruction in the rabbit model. In addition to the fact that we performed a partial tendon transfer rather than an autograft transplantation, we cannot be certain that the patellar tendon was placed intra-articularly and thus the measurements may not accurately reflect the tension applied through the transferred tendon. From our preliminary study, we decided it would be appropriate to compare two different environments for the healing of tendon transfer with and without a bone block. Another drawback of this model is the difference in the intraosseous length of the graft between the two groups. In order to reduce this, the drill hole in the tibia was located differently on the proximal articular surface as shown in Fig. 1. However, we found that implantation of the patellar tendon with a bone block provided more stable fixation than that without a bone block. Higher friction through the bone-to-bone contact indicated stronger fixation in group II in the immediate postoperative period. There was, however, no difference in the structural properties of graft fixation at 2 weeks after implantation. Thus the mechanical advantages of bone-to-bone fixation were no longer present at 2 weeks. Serial histological observations on the sites of tendon-to-bone healing showed progressive restoration of collagen-fibre continuity between bone and tendon. Perforating collagen fibres connecting tendon to bone appeared by 8 weeks. These fibres could be considered as Sharpey's fibres, which were first described by Sharpey and Ellis as the "perforating fibres" extending between periosteum and underlying bone at the site of insertion of tendon [2, 6]. Several studies showed the restoration of Sharpey collagen fibre continuity between bone and tendon in animal models [3, 8, 10, 11]. Another finding at the junction of the insertion of the tendon to bone was the formation of a layered structure of fibrocartilage by 12 weeks. This layered fibrocartilage structure has been described by several authors as a normal histological finding [2, 6, 7, 9, 12]. While the Sharpey perforating collagen fibres were seen within the intraosseous tunnel, the layered fibrocartilage was mainly around the entrance of the tendon into the tunnel. These findings may indicate the maturation of tendon-to-bone healing which can sustain a physiological load. Histological findings at 2 weeks did not show direct continuity between graft and tibia in both groups and thus there was not adequate strength of fixation at this time. In contrast, 12-week specimens of both groups showed sufficient organisation of the attachment of the graft to allow adequate mechanical strength. Rodeo et al. [11] reported that the strength of tendon-to-bone attachment increased until 12 weeks, at which time the bony attachment of the tendon was no longer the weakest link. The higher biomechanical values in the bone-to-bone healing group than in the tendon-to-bone healing group at 8 weeks correlated well with the histological findings. Definite bone-to-bone healing was noted in group II specimens while group I specimens showed only partial restoration of collagen fibre continuity between tendon and bone.

These histological and biomechanical results suggest that the healing of bone to bone is mature at 8 weeks, while the healing of tendon to bone is mature at 12 weeks. We recommend that the postoperative rehabilitation of patients who have undergone knee ligament reconstruction using a tendon graft without a bone block should be carefully managed in the immediate postoperative period to avoid undue tension at the site of the insertion of the graft.

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