Augmentation of tendon-bone healing by the use of calcium-phosphate cement

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The healing of a hamstring graft to bone is the weak link in the reconstruction of a cruciate ligament using this donor material. We therefore investigated the augmentation of healing at the tendon-bone interface using calcium-phosphate cement (CPC).

We performed semitendinosus autograft reconstructions of the anterior cruciate ligament on both knees of 22 New Zealand white rabbits. The interface between the grafted tendon and the bone tunnel for one knee was filled with CPC. Six rabbits were killed at the end of the first and second post-operative weeks in order to evaluate the biomechanical changes. Two rabbits were then killed sequentially at the end of weeks 1, 3, 6, 12 and 24 after operation and tissue removed for serial histological observation.

Histological examination showed that the use of CPC produced early, diffuse and massive bone ingrowth. By contrast, in the non-CPC group of rabbits only a thin layer of new bone was seen. Mechanical pull-out testing at one week showed that the mean maximal tensile strength was 6.505 ± 1.333 N for the CPC group and 2.048 ± 0.950 N for the non-CPC group. At two weeks the values were 11.491 ± 2.865 N and 5.452 ± 3.955 N, respectively.

Our findings indicate that CPC is a potentially promising material in clinical practice as regards its ability to reinforce the fixation of the tendon attachment to bone and to augment the overall effectiveness of tendon healing to bone.
Mechanical testing of the interfacial healing tissue. The Instron material testing machine model #1322 (Instron, Canton, Massachusetts) featuring a 50 kg load cell was used to detect the maximal tensile strength of the healing tissue. The test samples were dissected to the level of a bone-tendon-bone model, the bones of which were mounted at each end of the tubular mounts with cross pins and resin. The femur was fixed at 45° of flexion in order to align the bone tunnel along the direction of the testing force. Before beginning the stress loading, the suture fixing the grafted tendon to the LCL was removed so that the interface tissue would be the only material to be exposed to the pulling force. The original attachment of the grafted semitendinosus tendon to the tibia was allowed to remain. It was anticipated that the failure point would be situated at the site of the femoral bone tunnel. The tensile force was applied at a rate of displacement of 5.0 mm/s until the point was reached at which the tendon was pulled out of the femoral bone tunnel. The maximal level of tensile strength corresponding to this point was thus determined and the data were analysed statistically by the Wilcoxon signed-rank test.

Histological study of the interfacial healing tissue. Initially, the dissected knees were fixed in a neutralised formalin solution for a period of 72 hours after which the ossified material was decalcified for a period of one week using a mixed solution containing 20% sodium citrate and 50% formic acid. They were then embedded in paraffin and were sectioned perpendicular to the bone tunnel. The tissue was mounted on glass slides, deparaffinised and stained with haematoxylin and eosin for subsequent light microscopy.

Results
All the participating test rabbits tolerated the reconstructive procedure acceptably well. No wound infections were seen and no loss in body-weight was noted.

Histological and gross findings
One-week specimens. Histological examination of the CPC group (Fig. 1) showed that most of the tendon-bone interface was filled with CPC, although thin layers of fibrous tissue were observed between CPC fragments and adjacent to the surface of both the grafted tendon and the bone tunnel. In the non-CPC group, there was a collection of loose tissue in the interface consisting mainly of fibrous tissue.

Three-week specimens. Histological examination showed many growing bone islands within the CPC material some of which had formed directly on the surface of the bone and the tendon. There appeared to be a continuity between the bone and tendon. The healing tissue of the non-CPC group showed an increased production of extracellular collagen fibres which were distributed in a circular orientation around the grafted tendon. Those adjacent to the grafted tendon had an irregular orientation and were interwoven with the fibres of the grafted tendon. Collagen fibres attached to the bone tunnel were observed only occasionally.

Six-week specimens. Histological examination showed that the interface between the bone tunnel and grafted tendon was almost filled by new bone (Fig. 2). The fibres of the grafted tendon appeared to be firmly anchored onto the new growing bone. Some portions of the collagen fibres in

2.4 mm, was made in the proximal tibia just anterior to the medial collateral ligament (MCL) ending at the original point of insertion of the ACL on the intercondylar tibial spine. A femoral tunnel of the same size was made from the midline of the intercondylar notch ending just superior to the origin of the lateral collateral ligament (LCL). The semitendinous tendon was dissected and passed through the tibial and femoral bone tunnels and sutured to the LCL. The procedure was repeated for the other knee. The interface between the grafted tendon and the femoral bone tunnel of one knee was filled with 0.5 ml of CPC injected into the interface from a 2 ml syringe with a 19-gauge needle through the extrusion orifice of the femoral bone tunnel.

The limbs were not immobilised after completion of the surgical procedures and the rabbits were allowed to undertake normal activity within the cage. The results of a similar study indicated that after three weeks the intra-articular tunnel.

The Instron test.
the interface had matured to become organised into bundles.

**Twelve-week specimens.** Histological examination showed that most portions of the bone-tendon interface appeared to have been filled by new bone. Continuity between the collagen fibres of the grafted tendon and surrounding bone was seen. In the non-CPC group no formation of new bone was seen in the interfacial gap and even the native bone trabeculae appeared to have become thinner. An accumulation of adipose cells was found in the interface and in the bone-marrow space. These findings suggested an osteoporotic change.

**Twenty-four-week specimens.** Histological examination showed that the bone-tendon interface appeared to have healed completely as a result of bone ingrowth and continuity between the collagen fibres of the grafted tendon (Fig. 3). The surrounding bone had been remodelled and resembled Sharpey’s fibres.

A layer of newly-formed lamellar bone was observed in the bone tunnel.

**Mechanical testing.** Six specimens from the CPC group and six from the non-CPC group were tested for the mechanical strength of the bone-tendon union at both one and two weeks. All the specimens failed the test as a result of pullout of the tendon from the femoral bone tunnel. At one week in the CPC group the mean maximal tensile strength of the union was $6.505 \pm 1.333$ N and in the non-CPC group $2.048 \pm 0.950$ N (Table I) (Wilcoxon signed-rank test, $Z = -2.207$, $p = 0.027$, $p < 0.05$). At two weeks the mean maximal tensile strength in the CPC group was $11.491 \pm 2.865$ and in the non-CPC group $5.452 \pm 3.955$ N (Table II) (Wilcoxon signed-rank test; $Z = -2.201$, $p = 0.028$, $p < 0.05$).

<table>
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<tr>
<td>Mean</td>
<td>6.505 ± 1.333</td>
<td>2.048 ± 0.950</td>
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patients.9,14-16. The quadrupled hamstring tendon graft has fractures have been reported in approximately 2% of not unusual and kneeling may be uncomfortable. Patellar permanent weakening of the quadriceps by about 10%12,13 is chronic anterior knee pain after such reconstruction. Per-

Discussion
The autogenous bone-patellar tendon-bone graft is the most widely used graft for reconstruction of cruciate ligaments. Between 10% and 40%1,6,10,11 of patients report chronic anterior knee pain after such reconstruction. Permanent weakening of the quadriceps by about 10%12,13 is not unusual and kneeling may be uncomfortable. Patellar fractures have been reported in approximately 2% of patients.9,14-16 The quadrupled hamstring tendon graft has attracted wide popularity.17-22 The initial fixation and early healing appear to be inherent weaknesses of the hamstring-graft procedure, and therefore most surgeons, recommend a cautious programme of physiotherapy for periods of up to eight weeks.11,23

A method of augmenting the healing of the tendon to bone may improve the clinical results. The use of a fresh periosteal autograft24 and/or the administration of specific bone morphogenetic proteins25,26 have been reported.

In 1983, Brown and Chow27 reported that the application of a mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) powders in a diluted phosphate-containing solution led to the formation of hydroxyapatite. Despite its many advantages, there are still some practical problems associated with the use of CPC. These include the prolonged setting time of the material and its potential for dispersion on early contact with blood or aqueous media, substantially limiting its use for bone repair and reconstruction augmentation. Recently, a non-dispersive, faster-setting and high-strength TTCP/ DCPA-based CPC material has been developed in one of the author’s (JHCL) laboratories (The Centre for Biological Materials Research at the National Cheng-Kung University). A comparison between this non-dispersive CPC (nd-CPC) and a conventional TTCP/DCPA-based CPC (c-CPC) has been conducted and has shown that during immersion testing, there was a substantial difference in dispersion behaviour between the conventional CPC and the nd-CPC. The latter did not disperse in aqueous solution but even if it did it was in a paste form. Conventional CPC quickly dis-integrated in a simulated body fluid even after having set. The nd-CPC material has a higher rate of formation of apatite and also a denser structure than conventional CPC especially in the early stages. Relatively high levels of strength are achieved at an early stage of curing even when it is subjected to immersion in an aqueous medium. The 20-minute compression strength (CS) and diametral tensile strength (DTS) of this new material were relatively high (41.6 and 8.1 Mpa, respectively). The maximum levels (CS = 103 MPa and DTS = 16.3 Mpa) were attained after four to eight days from the time of immersion in a simulated body fluid.5,9

We have shown that the use of CPC augments healing at the tendon-bone interface in this rabbit model as seen histologically by increased bone ingrowth into the tendon and mechanically with increased tensile strength on pull-out tests. The development of new forms of CPC with greater strength may further enhance the process.

Table II. The maximal tensile strength (N) in both groups at two weeks (Wilcoxon signed rank test \(Z = -2.201, p = 0.028, p < 0.05\))

<table>
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References