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Peri-graft bone mass and connectivity as predictors for the strength of tendon-to-bone attachment after anterior cruciate ligament reconstruction

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ABSTRACT

The present study was designed to compare peri-graft bone mass and microarchitecture with tendon-to-bone (T–B) attachment strength after anterior cruciate ligament (ACL) reconstruction in a rabbit model. Surgical reconstruction using digital extensor tendon in bone tunnel was performed on 58 rabbits. Forty-two of the 58 rabbits were sacrificed at week 0, 2, 6 and 12 after operation respectively. The femur–graft–tibia complexes were harvested for pQCT and micro-CT examination to characterize the spatiotemporal changes of peri-graft bone in T–B healing in conjunction with histological examination. The remaining 16 rabbits were euthanized at week 6 and 12 postoperatively (i.e. 8 rabbits for each time point) for pull-out test after micro-CT examination to investigate the relationship between the T–B attachment strength and peri-graft bone mass/microarchitecture. Peri-graft BMD, BV/TV and connectivity was significantly lower at week 6 than those at time zero although there were no significant changes detected in the first 2 postoperative weeks. In addition, peri-graft bone mass and connectivity was significantly lower on the tibial side than those on the femoral side; and osteoclasts accumulated on the surface of peri-graft bone. Grafted tendon was prone to be pulled out from the tibial tunnel with bone attachment; the weakest point of the complexes shifted from the healing interface at time zero to peri-graft bone at week 6 after operation. With reverse of peri-graft bone at week 12 postoperatively, the weakest point shifted to the intra-osseous tendinous portion. The stiffness of T–B attachment correlated with peri-graft BV/TV ($r^2 = 0.68$, $p = 0.001$) and connectivity ($r^2 = 0.47$, $p = 0.013$) at week 6 after operation. T–B healing was a highly dynamic process of emergence and maintenance of peri-graft bone. T–B attachment strength was in relation to peri-graft bone mass and connectivity after ACL reconstruction. The measurement of peri-graft bone should be useful to monitor the quality of T–B healing and guide the postoperative rehabilitation.

Introduction

Anterior cruciate ligament (ACL) rupture is one of the most common knee injuries in sports medicine. Surgical reconstruction using tendon graft in bone tunnel is frequently performed to restore knee integrity and stability [1]. Tendon–bone (T–B) healing and its attachment strength is the deterministic factor for the outcome of ACL reconstruction [2]. Prediction of T–B healing and its attachment strength is an issue of major clinical importance for the guidance of postoperative rehabilitation and return to activity.

Grassman et al. found that T–B healing in trabeculae-rich femoral tunnel was better than in the marrow-rich tibial tunnel [3]. Recently, it was reported that a decrease of T–B attachment strength was accompanied by bone mineral loss within 6 weeks postoperatively in a phalangeal bone tunnel of canine [4,5]. Those findings indicated the potential relationships between the quality and quantity of peri-graft bone and T–B healing.

Bone mineral and microarchitecture at a native tendon/ligament-to-bone attachment site play a role in load transfer from tendon/ligament-to-bone [6–10]. It was revealed that bone mineral gradient across ligament-to-bone (L–B) attachment corresponded to regional mechanical properties [6]. Loss of bone mineral at L–B attachment site resulted in impairment of L–B mechanical properties [9,10]. Recently, it was observed that the force of Achilles tendon was transmitted through anisotropic trabeculae to plantar fascia [7]. Changes of microarchitecture of trabeculae indicated the initiation of disorders at T–B attachment site [8]. Naturally, there are no sites in human, where a tendon or ligament goes into a bone tunnel; therefore, there is no native situation analogous to the attachment site of grafted tendon-to-tunneled bone [11]. It remains unknown whether the strength of T–B attachment inside a tunnel was related to bone mineral and microarchitecture in the vicinity of a grafted tendon after ACL reconstruction.

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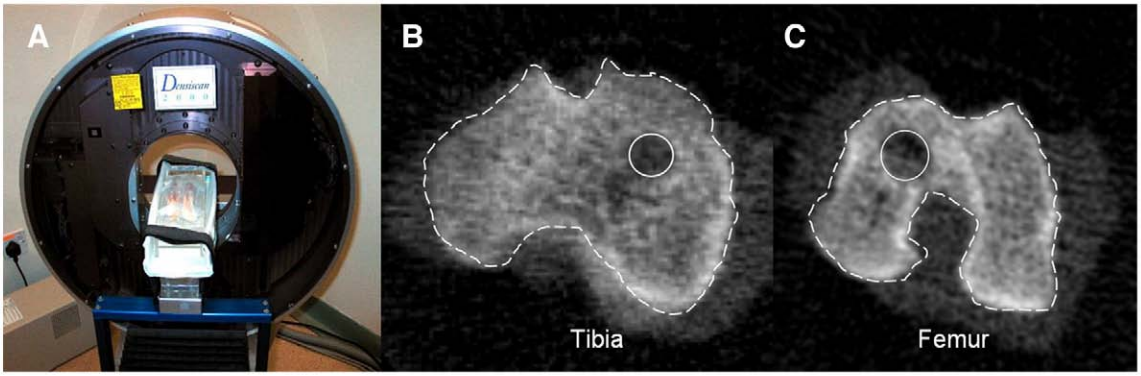

Fig. 1. pQCT scan and examination. (A) Distal femur and proximal tibia were scanned perpendicular to the long axis of limb; (B and C) One representative slice in the middle portion of bone tunnel was selected from the tibial (B) and from the femoral side (C) for analysis. On each slice, bone mineral density (within the dotted line) and bone tunnel area (within circle) were measured.

The present study was designed to investigate spatiotemporal changes of BMD and bone microarchitecture in the vicinity of a grafted tendon postoperatively using both pQCT and micro-CT, and compare radiological findings with the pull-out strength of the tendon graft in an ACL reconstruction rabbit model. We hypothesized that the pull-out strength of T–B attachment inside a tunnel related to peri-graft bone mass and microarchitecture after ACL reconstruction. Accordingly, the findings of the present study would provide useful non-invasive imaging modalities for monitoring healing process and quality of T–B healing inside the tunnel after common ACL surgical reconstruction in sports medicine.

Materials and methods

Study design

This experiment was approved by the Research Ethics Committee of the authors' institute (Ref No. CUHK06/004/ERG). Fifty-eight skeletally-mature female New Zealand white rabbit (26-week-old; weight: 3.5–4.0kg) were used in the present study. Bilateral ACL reconstruction was performed. One reconstructed knee was randomly selected for the subsequent examination in the present study. The contralateral knee was treated by local application of calcium phosphate cement for enhancement of peri-graft bone healing and T–B attachment strength, and the related findings were reported separately [12].

42 of 58 rabbits were sacrificed at week 0, 2, 6 and 12 after operation respectively for pQCT and micro-CT examination to characterize the spatiotemporal changes of peri-graft bone in T–B healing in conjunction with histological examination. 6 rabbits were sacrificed at time zero (week 0 after operation) for pQCT and micro-CT evaluation and served as the baseline for comparison. The other 36 rabbits were sacrificed at 2, 6 and 12 weeks respectively after surgery (i.e. 12 rabbits for each time point). At each time point, 6 samples were subjected to pQCT evaluation and decalcified histological examination; the other 6 samples were used for micro-CT evaluation and followed by undecalcified histological examination.

The rest of 16 rabbits were euthanized at week 6 and 12 respectively (i.e. 8 rabbits for each time point). The samples were processed for biomechanical test after micro-CT evaluation to investigate the relationship between the strength of T–B attachment and peri-graft bone mass and microarchitecture. The failure mode was judged by both gross and microscopic examination after the test.

Animal surgery

ACL reconstruction rabbit model was established according to an established protocol by Wang et al. [13]. In brief, the rabbits were operated under general anesthesia with 10% ketamine/2% xylazine (Kethalar, 1mL:1mL) and maintained sedation with 2.5% sodium phenobarbital injected intravenously (Sigma Chemical Co., St. Louis, Mo, USA). The medial parapatella arthrotomy was performed to expose the knee joint. Patella was then dislocated and infrapatella fat pad was removed to expose the joint cavity. ACL was excised and transverse meniscal ligament was also removed. The long digital extensor tendon graft was harvested and graft preparation was done by removing the attached muscle and passing the holding sutures through each end of the tendon graft in an interdigitating whipstitch fashion. Femoral and tibial tunnels were created through the footprint

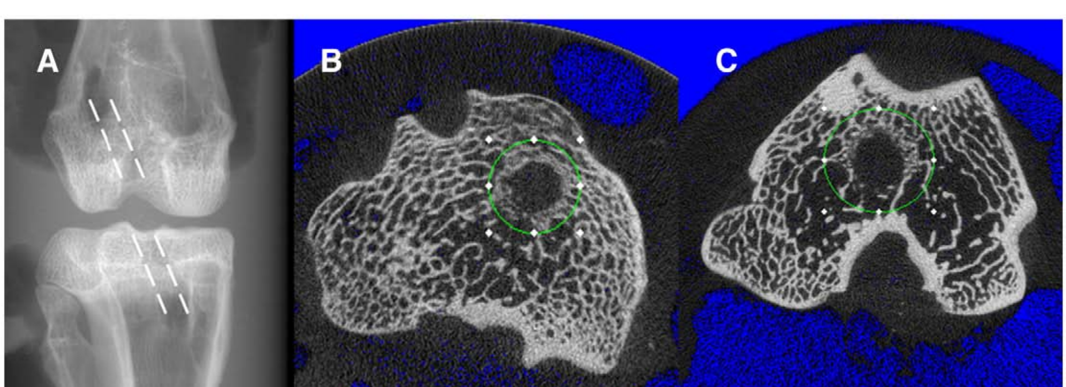

Fig. 2. Micro-CT scan and analysis. (A) The femoral and tibial tunnel, showed by white dotted line, were scanned perpendicular to the long axis of limb; (B and C) The region of interest of 6mm diameter was defined to cover bone tunnel on both the tibial (B) and femoral side (C).