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Original Article

An animal model of reconstruction of single femoral tunnel with single bone bi-quadruple ACL and internal fixation

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Abstract

We introduced several variables in an animal model of anterior cruciate ligament (ACL) reconstruction to determine the best parameters for surgery in humans. We divided 130 LYD pigs into two groups depending on whether the femoral tunnel goes through the medial tibial tunnel or through the medial fossa of the knee joint. Each subgroup was further divided. Four weeks after surgery the knee specimens were examined for passive flexion and extension test. No group showed a creep effect. In the biomechanical tests, we recorded maximal strength, maximum load, and stiffness parameters. The 100° + 1.0 mm, 1.5 mm, and 2.0 mm positions of the tibial tunnel group, and 10.5 (1.5) + 1.0 mm, 1.5 mm, and 2.0 mm positions of the knee joint cavity group had better biomechanical effects, histocompatibility and revascularization in ACL reconstruction. Overall, these results demonstrated significant differences in the effectiveness of ACL reconstruction based on several surgical parameters, which should contribute to establishing a gold standard for ACL surgery in patients.

Keywords: Acl Reconstruction, Femoral Tunnel, Internal Fixation, Biomechanics, Histocompatibility, Revascularization

Introduction

Arthroscopic anterior cruciate ligament (ACL) reconstruction is still a challenging surgery. First, it is critical to select the appropriate autologous or artificial graft materials and fixtures[1]. Then, the surgical team needs to choose a safe and effective femoral tunnel direction and path, and accurately locate the ACL positions in the intercondylar space to avoid the "pendulum or wiper effect". Since the grafts and fixation materials can cause wear and cut into the tunnel[2], keeping a tight compression of the grafts and bone tunnel can effectively prevent the femoral bone from oozing synovial fluid into the bone tunnel. Strengthening the interface were friction occurs and promoting healing of the graft and tunnel will result in a strong initial stability and long-term joint fixation. So far, there is no recognized "gold standard" for femoral fixation during ACL reconstruction. Armed police officers and soldiers are prone to partial or complete ACL rupture due to fatigue or acute high-speed energy impact, resulting in instability or fear of walking. A mistake in ACL reconstruction surgery or in the selection of the graft material may result in surgical failure, unsatisfactory recovery, or a second surgery, which causes long-term pain to many patients[3].

The aim of this study was to establish an animal model of ACL injury on the basis of single-bundle single-tunnel ACL reconstruction. We explored the location of different femoral tunnel positioning paths, close to or far from the normal femoral ACL location, different femur tunnel length and direction, and different femoral tendon graft fixation devices. These studies are expected to provide some clarity to the "gold standard" for femoral tunnel ACL reconstruction and fixation.

Materials and methods

Generation of a pig model of ACL injury

We used 130 LYD pigs as the experimental group (7.2±0.4 months, 22.4±1.5 kg). Ten LYD pigs without ACL fracture or reconstruction served as the control group (7.3±0.3 months,

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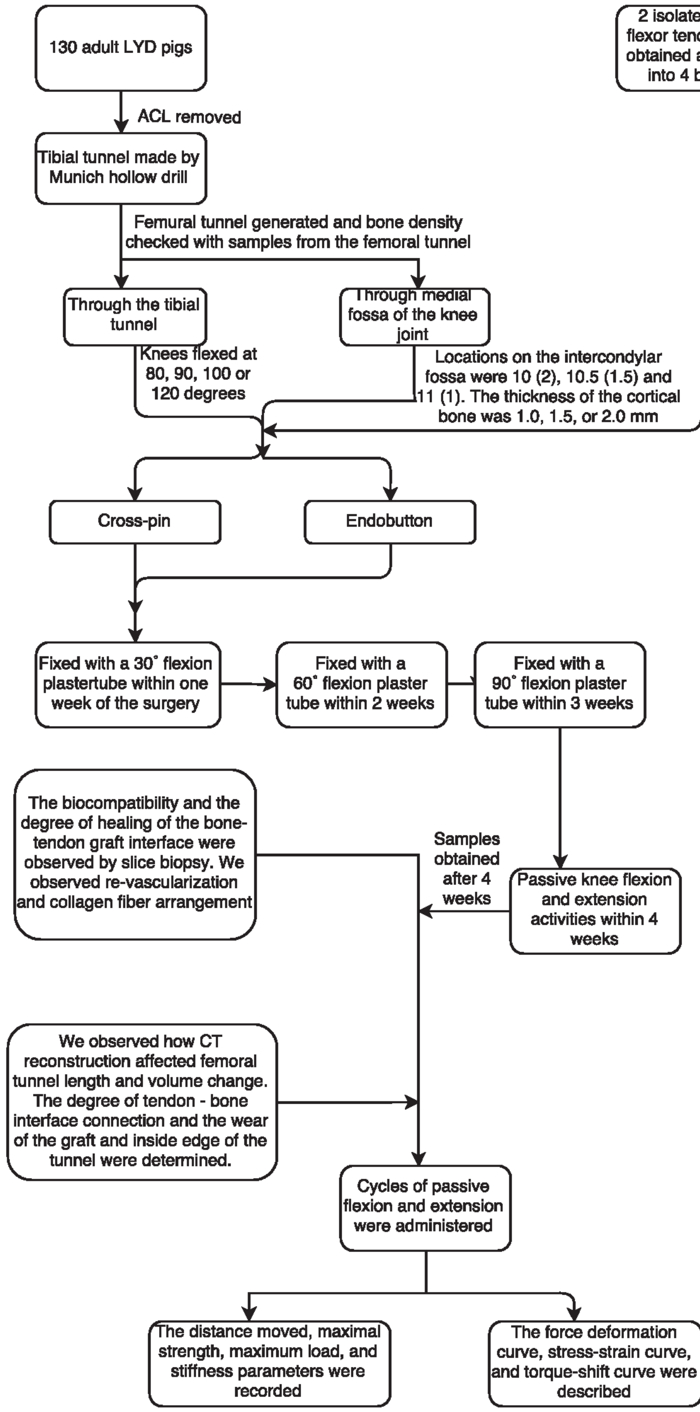

Figure 1. Schematic summary of the study.

23.1±1.8 kg). All experimental animal procedures were carried out in accordance with the European Directive 2010/63/EU. The experiments were performed after keeping the animals for one week in the lab. Generation of the ACL model: 3% pentobarbital sodium (1 mL/Kg) was used to anesthetize the animals in the supine position through abdominal injection. Skin was disinfected and covered with sterile towels 15 cm above and below the knee line. Preoperative tendon pre-tension was carried out to reduce tendon viscoelasticity. The medial patellofemoral approach was used to dissect the articular cavity and the patella was dislocated. The flexion of the knee was sufficient to expose the intercondylar fossa, and the partial patellar fat pad was removed until the tibial end of the ACL was exposed. We cut the connection posts of the ACL with the femur and tibia, and all the ligaments and synovial tissues in between. The anterior drawer test (ADT) was positive.

Research methods

The 130 LYD pigs were divided into two groups according to whether the location of the femoral tunnel goes through the medial tibial tunnel or the medial fossa of the knee joint. The tibial tunnel group was divided into subgroups based on the knee flexion at 80°, 90°, 100°, and 120°. The knee joint group was divided into three subgroups based on the locations on the intercondylar fossa, which were 10 (2) o'clock, 10.5 (1.5) o'clock and 11 (1) o'clock, respectively. That is, the top of the femoral condyle fossa was set as 12 o'clock, and the right knee was 10 o'clock (the direction of left knee femoral tunnel was 2 o'clock), 10.5 o'clock (10:30 o'clock) and 1.5 (1:30 o'clock for left knee), 11 o'clock (1 o'clock for left knee) respectively; Each subgroup was further divided into three groups according to the thickness of the posterior wall of the reserved femoral tunnel. The thickness of the cortical bone was 1.0, 1.5, or 2.0 mm. Each femoral tunnel approach corresponds to the lateral blocker and EndoButton tendon graft fixation methods. The tibial side can be fixed with a biodegradable interface nail/transfix. The subjects were fixed with a 30° flexion plaster tube within one week of the surgery, a 60° flexion plaster tube within 2 weeks, and a 90° flexion plaster tube within 3 weeks. At four weeks, they were placed on a walking or running exercise machine (Elbow/ankle rehabilitation machine, canwell, Zhejiang, China) for passive knee flexion and extension activities. After four weeks, the knee specimens were taken for passive flexion and extension test (Figure 1).

Observation parameters

The sensor (Andcn, Guangzhou, China) was placed proximal to the femoral tendon graft. Without any load, the tendon had 20 cycles of passive flexion and extension, with a force of 89 Newton for 5 min to observe the creep effect of the tendon graft in the femoral tunnel. We had 12 subgroups in the tibial tunnel test group and 9 subgroups in the knee joint cavity group. No group was observed with a creep effect.

Biomechanical tests

The tendon grafts were washed with saline and the flexion of the knee joint was maintained at 30° for the uniaxial tension test. The tendon grafts were pulled at a rate of 150 mm/min (Multisample oral bio-medical materials biomechanical testing machine, Micoforce, China). The maximal strength, maximum load, and stiffness parameters were recorded.

Histology

The subjects were sacrificed using air embolism method and specimens of the knees were collected four weeks after Biomechanical tests. The biocompatibility and the degree of healing of the bone-tendon graft interface were observed by slice biopsy. We observed re-vascularization and collagen fiber arrangement and how CT reconstruction affected femoral tunnel length and volume change. The degree of tendon - bone interface connection and the wear of the graft and inside edge of the tunnel were determined. Four weeks after surgery, the cells in the tendon graft were completely necrotic and infiltrated, and the tendon-bone interface was continuous, showing loose tissue, abundant blood vessels, and cells.

Statistical methods

SPSS 20.0 software was used for statistical analysis. Quantitative data were indicated using mean ± standard deviation. An independent sample t-test was used for comparison between two groups. Multi-group comparison was conducted using single-factor ANOVA analysis. Comparison of two pairs was conducted using LSD-t test; $p<0.05$ suggested that the difference was statistically significant.

Results

Analysis of the creep effect

There were 12 subgroups in the tibial tunnel test group and 9 subgroups in the knee joint cavity group. No obvious creep effect was observed in any of them.

Analysis of the biomechanical parameters

The tibial tunnel groups were as follows: 80° and 1.0 mm A1, 80° and 1.5 mm A2, 80° and 2.0 mm A3, 90° and 1.0 mm A4, 90° and 1.5 mm A5, 90° and 2.0 mm A6, 100° and 1.0 mm A7, 100° and 1.5 mm A8, 100° and 2.0 mm A9, 120° and 1.0 mm A10, 120° and 1.5 mm A11, and 120° and 2.0 mm A12. The knee joint cavity groups are as follows: 10 (2) 1.0 mm B1 group, 10 (2) 1.5 mm B2, 10 (2) 2.0 mm B3, 10.5 (1.5) 1.0 mm B4, 10.5 (1.5) 1.5 mm B5, 10.5 (1.5) 2.0 mm B6, 11 (1) 1.0 mm B7, 11 (1) 1.5 mm B8, and 11 (1) 2.0 mm B9. The maximal strength, maximum tensile load, and stiffness of A7 - A9 and B4 - B6 were close to the control group and were significantly higher than the other subgroups ($P<0.05$) (Table 1).

Distance of tibia relative to femur

The relative distance was 4.2±0.9 mm in A1, 4.0±1.2 mm in A2, 3.8±0.7 mm in A3, 4.3±1.1 mm in A4, 4.4±1.3 mm in A5, 4.6±1.5 mm in A6, 3.2±0.5 mm in A7, 3.0±0.6 mm in A8, 3.5±0.8 mm in A9, 4.4±1.3 mm in A10, 4.1±1.2 mm in A11, and 4.4±1.5 mm in A12. The relative distance was 4.6±1.3 mm in B1, 4.7±1.4 mm in B2, 4.8±1.6 mm in B3, 3.5±0.6 in B4, 3.6±0.7 mm in B5, 3.7±1.0 mm in B6, 4.2±1.3 mm in B7, 4.4±1.5 mm in B8, and 4.8±1.7 mm in B9. The control group had a mean distance of 3.6±0.8 mm. The forward distances of A7-A9 and B4-B6 were similar to the

Table 1. The comparison of maximal strength, maximum tensile load and stiffness.

Group	Maximum strength (MPa)	Maximum tensile load (N)	Stiffness (N/m)
Control	56.2 ± 6.9	354.7 ± 45.7	152.5 ± 32.3
A1	37.6 ± 4.5	205.9 ± 25.6	92.5 ± 12.4
A2	40.5 ± 4.8	223.2 ± 22.3	106.9 ± 11.5
A3	42.3 ± 4.9	246.5 ± 18.7	115.4 ± 13.2
A4	30.2 ± 3.6	176.8 ± 16.5	76.8 ± 6.8
A5	31.5 ± 3.3	165.9 ± 14.5	81.2 ± 7.2
A6	32.6 ± 3.5	184.2 ± 13.8	75.5 ± 7.5
A7	47.2 ± 5.2	289.5 ± 20.2	131.2 ± 16.9
A8	48.5 ± 5.3	302.6 ± 26.5	135.6 ± 15.7
A9	51.1 ± 5.5	313.5 ± 23.9	141.2 ± 18.3
A10	41.5 ± 4.4	232.6 ± 24.4	105.6 ± 14.4
A11	38.9 ± 3.8	212.5 ± 21.2	112.4 ± 12.6
A12	37.6 ± 3.9	245.2 ± 19.5	126.8 ± 13.3
B1	35.9 ± 4.2	256.4 ± 1 5.6	92.5 ± 12.6
B2	37.6 ± 4.3	275.4 ± 16.7	93.6 ± 13.3
B3	38.4 ± 4.4	289.3 ± 17.2	95.4 ± 14.5
B4	45.6 ± 4.6	312.5 ± 21.3	126.9 ± 15.9
B5	47.8 ± 4.7	302.6 ± 22.4	128.4 ± 16.6
B6	49.7 ± 4.8	323.4 ± 23.6	130.5 ± 17.2
B7	32.1 ± 3.5	232.5 ± 12.5	76.8 ± 10.3
B8	31.6 ± 3.6	212.4 ± 11.6	78.2 ± 11.5
B9	30.5 ± 3.3	225.6 ± 12.4	79.5 ± 12.2

control group, and were significantly smaller than the other subgroups ($p<0.05$).

Analysis of histological observations

Four weeks after surgery, the cells in the tendon graft were completely necrotic and infiltrated and the tendon-bone interface was continuous, showing loose tissue, abundant blood vessels and cells.

Discussion

The femoral and tibial tunnels are different. The femoral tunnel has isometric regions on uneven femoral condylar fossa with curvature. It is relatively small, so the location of the femoral tunnel has a big impact on the change of length of tendon grafts in knee flexion and extension, especially the impact of tension[4]. If the tension is too small, it will not maintain the stability of the knee. If it is too large, it will limit the physiological activities of the knee, or even cause ruptures. Both can increase the contact stress of the articular cartilage, leading to cartilage degeneration, affect the remodeling of collagen fibers, prevent the formation of an early complete fiber - cartilage connection, and affect the formation of tidal mineral layer[5].

After ACL reconstruction, the tendon grafts undergo necrosis, cell proliferation, revascularization, cell neogenesis, and neoplastic remodeling at the femoral tunnel[6]. An ideal ACL reconstruction needs to satisfy an important biomechanical principle: regardless of the degree of flexion and extension of the knee, the anterior cruciate ligament should be kept at the same length as the graft within the physiological activity in the knee[7]. Maintaining the tendon graft at the same length in the knee during passive flexion and extension is largely determined by the location of the femoral tunnel. The location of the femoral tunnel and its fixation determine the initial, middle, and long-term effects of ACL reconstruction[8-10]. However, the wrong position for femoral tunnel reconstruction results in an imbalance in tension on the tendon graft, causing poor internal fixation, tendon graft relaxation, or wear, which makes the isometric reconstruction of ACL very difficult[11]. When the femoral tunnel location is too high or too far forward the change in length of the graft during knee joint activities increases. The tendon graft withstands excessive tension, which increased tissue strain. The excessive graft extension can lead to permanent extension of the tendon graft, causing joint instability[12]. The femoral tunnel is too far on the back compared to the original ACL attachment site. When the thickness of the posterior wall of the cortical bone is not enough, the lateral block nail or Endobutton can be pulled out of the femoral tunnel and lead to bone perforation at the back of the tunnel[13].

The breakthrough of this study consists in establishing the first animal model of ACL reconstruction. We established several femoral tunnels with different pathways according to the position and knee flexion angle, different sites of the intercondylar fossa, and different femoral tunnel wall thickness. The transverse tendon nail and Endobutton plate were used to fixate the tendon graft. Biomechanics, CT reconstruction, and slices embedded in collodion were used to comprehensively evaluate biomechanics, imaging, and gross histopathology. We show that the tibial tunnel at 100°+1.0 mm, 1.5 mm, and 2.0 mm, and knee joint cavity at 10.5 mm (1.5)+1.0 mm, 1.5 mm, and 2.0 mm have good biomechanical effects, histocompatibility, and revascularization in ACL reconstruction.

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