Establishment of near and non isometric anterior cruciate ligament reconstruction with artificial ligament in a rabbit model

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ABSTRACT

Background: Tunnel position decide the isometry of graft attachment in synthetic anterior cruciate ligament (ACL) reconstruction. Near-isometric tunnel position may have advantage in graft integration and knee function in ACL reconstruction (ACLR) with polyethylene terephthalate (PET) ligament. Few studies focused on tunnel position isometry when conduct ACLR with an animal model. This study aimed to establish a preclinical rabbit model of near and non isometric ACLR with PET ligament and investigate the advantage of near-isometric ACLR compared to non-isometric ACLR.

Methods: Nine hind limbs of rabbit were used in tunnel position study. Two femoral (anatomic, nonanatomic) tunnels and three tibial (anterior, middle, posterior) tunnels were used to measure tunnel position isometry during knee full range of motion. The tunnel position combination with minimal isometry was considered as near-isometric tunnel position. Then, 48 rabbits divided into two groups were conducted near or non isometric ACLR with PET ligament with graft fixation angle of 30° and constant tension of 5N. PET ligament isometry, range of motion (ROM) restriction, knee laxity were recorded after operation and followed up with macroscopic observation, microcomputed tomography (micro-CT) analysis, histology assessment and biomechanical test at 4 and 8 weeks postoperatively.

Results: The tunnel combination with minimal isometry was femoral anatomic position and tibial posterior position (5.19° ± 1.78°) and considered as near-isometric tunnel position. ROM restriction were observed in non-isometric group (22.50° ± 14.14°) while none in near-isometric group. However, no ROM restriction observed at 8 weeks in both group. Knee laxity compared to contralateral knee were better in near-isometric group than non-isometric group (stable/slack/total 10/2/12 VS 3/9/12, p = 0.012) at 8 weeks postoperatively. Superior PET ligament integration were also observed in near-isometric group through macroscopic observation, micro-CT analysis, histology assessment at both 4 and 8 weeks. The failure load in the Near-Isometric group at 8 weeks were higher than timezero reconstruction with statistical difference (156.8N ± 25.98N vs.102.6 ± 22.96N, p = 0.02).

Conclusion: A rabbit model of ACLR based on tunnel position isometry was successfully established in this study. The near-isometric tunnel position in rabbit model was femoral anatomic position and tibial posterior position. A near-isometric ACLR with PET ligament did not cause ROM restriction and had a better graft integration and follow-up stability than non-isometric ACLR with ROM restriction.

The Translational Potential of this Article: The study demonstrate the establishment of near-isometric tunnel position and non-isometric tunnel position with significant difference of ROM restriction and graft-bone integration. The described tunnel positions with differential isometry in a rabbit ACLR provides a reproducible and translational small animal model and enables preclinical research between tunnel position isometry and its affection on variable grafts, graft integration and knee function.
Figure 1. a. Laboureau described the isometric femoral tunnel position. b. The simulated knee condylar circle and geometric femoral tunnel position in the X-ray image of the rabbit knee. c. The non-anatomical tunnel position in a rabbit knee specimen. The red line (a, b, c) indicates the Blumensaat's line. The blue line (a, b, c) shows 60% of the anteroposterior length of the condyle. d. Geometric tunnel placement in the rabbit knee after ACL resection. PCL: Posterior cruciate ligament. Starred: Tibial enthesis of ACL. (For interpretation of the references to /colour in this figure legend, the reader is referred to the Web version of this article.)

Figure 2. a. The tibial tunnel positions: anterior (Ti-An), middle (Ti-Mi), posterior (Ti-Po). b. Femoral tunnel positions: anatomical (Fe-A), non-anatomical (Fe-N).
1. Introduction

Isolated anterior cruciate ligament (ACL) tears remain a common orthopedic injury with an annual incidence of 68.6 per 100,000 person years [1]. Single bundle ACL reconstruction (ACLR) has become one of the most routine treatment strategies that aim to restore knee stability and mobility [2]. Current graft options to replace torn ACLs are autologous grafts, allografts and synthetic grafts [3]. The tunnel position is one of the most critical considerations in successful single bundle ACLR. However, controversies [11] exist concerning the isometry (distance change of graft attachment/tunnel position during knee motion) of differential tunnel positions and how the tunnel position isometry affects the surgical outcomes. In this study, we aimed to establish an animal model to determine the optional tunnel positions in ACLR based on tunnel position isometry that can be used for further preclinical research studies.

Over the last decade, the development of tibial-independent drilling techniques using the anterolateral portal from the classical trans-tibial technique has allowed surgeons to adopt a more anatomical placement of the femoral tunnel that is beneficial in restoring normal ACL function [4–6]. However, it has been widely reported that the anatomical tunnel position has inconsistent isometry (length change of tunnel position during knee full range of motion) with variable values ranging from 0.7 to 13.4 mm [7–14] during knee motion, whilst the ideal graft length change agreed by most surgeons is <2 mm [7–10]. This acceptable length change is similar to the normal change in ACL length with passive knee motion (5%–6%, 2.5 mm) [10]. Several studies have reported that the ideal isometric tunnel position combination of the anatomical footprint is the anterior tibial point combined with the anterosuperior femoral point [8,11–14]. However, in some reported cases [12–14] the length change is >2 mm.

The non-anatomical but isometric femoral tunnel position was initially described by Laboureau and has been previously used for PET ligament ACLR [16]. PET ligaments are the most commonly used

Figure 3. a. The change in length change from the tibial cortex to the distal nodule (arrow) was inverse to the intra-articular tunnel position length change. b. The length change measurement set with a consistent coronal plane and suture strain. c. Electronic Vernier calipers.

Table 1
Initial length and changes of different tunnel position combinations during knee full range of motion.

| Tunnel position combinations | Initial distance (135°) (mm) | Length change (90°) (mm) | percentage(%) | Length change (45°) (mm) | percentage(%) | Length change (10°) (mm) | percentage(%) |
|-----------------------------|------------------------------|-------------------------|---------------|-------------------------|---------------|--------------------------|---------------|
| Fe–N, Ti–Po                 | 9.82 ± 1.05                  | -0.63 ± 0.20            | -6.30 ± 1.81  | -1.14 ± 0.37            | -11.53 ± 3.45 | -1.64 ± 0.42             | -16.69 ± 4.27 |
| Fe–A, Ti–Po                 | 9.10 ± 0.92                  | -0.15 ± 0.15            | -1.70 ± 1.54  | -0.22 ± 0.22            | -2.45 ± 2.16  | -0.48 ± 0.18             | -5.19 ± 1.78  |
| Fe–N, Ti–Mi                 | 11.18 ± 1.10                 | -0.87 ± 0.38            | -7.75 ± 3.41  | -1.50 ± 0.43            | -13.35 ± 3.68 | -2.22 ± 0.35             | -19.92 ± 2.79 |
| Fe–A, Ti–Mi                 | 10.30 ± 0.91                 | -0.28 ± 0.19            | -2.76 ± 1.25  | -0.55 ± 0.20            | -5.25 ± 1.85  | -0.98 ± 0.30             | -9.36 ± 2.56  |
| Fe–N, Ti–An                 | 12.49 ± 1.11                 | -0.84 ± 0.30            | -6.62 ± 1.96  | -1.73 ± 0.34            | -13.89 ± 2.19 | -2.72 ± 0.43             | -21.94 ± 2.24 |
| Fe–A, Ti–An                 | 11.45 ± 0.95                 | -0.46 ± 0.23            | -3.96 ± 1.98  | -0.97 ± 0.30            | -8.43 ± 2.43  | -1.52 ± 0.48             | -13.89 ± 3.43 |

Figure 4. The length changes in the tunnel position combinations during a full range of knee motion.
synthetic grafts [29] and are much stiffer than natural ligaments with length changes of less than 1 mm [21]. PET ligaments are impacted more by tunnel position isometry than autografts or allografts. Laboureau [15] found the femoral isometric point is located anterior to the anatomic footprint region, at approximately 60% of the anteroposterior length of the condyle when measured on a line parallel to the Blumensaat’s line. Recent computerized studies [17,18] have partially supported this viewpoint showing that an isometric region in the femoral sagittal condyle and the anterior non-anatomical point have better isometry compared to the anatomical point whilst individual differences exist. These studies focused more on the femoral tunnel position rather than the tibial position [8,11] and demonstrate that the femoral point option is more effective to the isometry of ACLR.

The tibial tunnel position also affects the isometry of ACLR. Several studies [7,8,12,13] have reported the anterior region of the tibial ACL footprint as the most isometric tibial tunnel position rather than the centric or posterior regions when matched with the anatomical femoral tunnel position. Other studies have focused on an “over the top” femoral tunnel position, however, a more posterior tibial tunnel position is recommended from the isometry [17,18] standpoint using the traditional transtibial technique.

The reported isometry of ACLR tunnel position varies from different studies and may be due to individual differences and methods. To date,
the effect of isometry of the ACLR tunnel position on the mature graft, graft-bone integration and rehabilitation are not well known. Only one study has previously used a small animal model to investigate the basis of tunnel position isometry. Ma et al. established [19] a rat model with a predictable ACLR graft strain and conducted a study [20] to compare high and low force ACLR. The animal model was based on graft strain and partly indicated that an isometric ACLR is beneficial for graft-tunnel osseointegration. However, further studies are needed to investigate the critical value of isometry of ACLR and the effects of different isometry on graft-bone integration, postoperative knee laxity and rehabilitation. A robust and translationally relevant animal research model of ACLR based on isometry is needed for preclinical research studies.

Nearly isometric ACL reconstruction [30] is a state that the graft placed in a minimal isometry that less than 2 mm intraoperatively. A near-isometric reconstruction would also be viable in an animal model with minimal isometry intraoperatively but the length change criteria would be relevant to the size of the animal. In this study, we describe a rabbit model that was used to investigate the isometry of different tunnel position combinations for ACLR. Non-anatomical and anatomical femoral tunnel positions paired with anterior, middle and posterior anatomical tibial tunnel positions were placed and each of the six pairs of tunnel position isometry was measured. We provisionally compared graft-bone integration between the minimal isometry (as near-isometric) tunnel position and the non-isometric tunnel position with PET ligament ACLR. We used this approach to test the hypothesis that reproducible near-isometric tunnel position combinations exist in a rabbit ACLR model.

2. Methods

All animal experimental protocols were approved by the Animal Care and Experiment Committee of Shanghai Fudan University. Nine male New Zealand white rabbits (skeletally mature, weighing 2.5–3.5 kg) with healthy and intact hind limbs were used for the tunnel position isometry study. One randomly chosen lower limb was used in each rabbit. Two combinations of tunnel positions with significantly different isometry were selected, one with minimal isometry as the near-isometric group and the other as the non-isometric group. Forty-eight skeletally mature male New Zealand white rabbits that had been assigned to the two experimental groups underwent ACL reconstruction with a PET ligament for the right knee. Graft isometry and the knee range of motion were recorded after surgery. At each follow-up, the range of knee motion and knee laxity were recorded in all rabbits. Micro-CT and histology analysis were performed in eight rabbits and biomechanical tests were performed in four rabbits in each group. Additionally, four contralateral intact knees were used for normal biomechanical tests and another 4 intact knees were used to test the time zero reconstruction biomechanical properties after surgery.

2.1. Tunnel position placement and isometry

General anesthesia was induced using Xylazine HCl (0.2 ml/kg) subcutaneously injected into the lower back followed by propofol controlled injection through the ear vein. After anesthesia and skin preparation, rabbits were fixed in the supine position on a flat board and a routine 4 cm incision was made medial to the patella tendon and the ACL exposed following patellar subluxation. The ACL was removed from the enthesis with a sharp blade to visualize the tibial and femoral footprint under the rabbit knee with maximum flexion.

The non-anatomical femoral tunnel point was made as previously described by Laboureau [15] at approximately 60% of the anteroposterior length of the condyle (Fig. 1a) when measured on a line through the center of the circle and parallel to the Blumensaat’s line. In our pilot study, we found that the rabbit knee had a similar simulated circle and intercondylar line from the lateral view of the knee joint under

Table 2

| ROM restriction | Time Zero | 4th Week | 8th Week |
|-----------------|-----------|----------|----------|
|                 | Number    | Angle    | Number   | Angle    | Number   | Angle    |
| Near-Isometric  | None      |          |          |          |          |          |
| Non-Isometric   | 23/24     | 22.50 ± 14.14 | 3/12     | 8.75 ± 6.29 | 0/12     |          |

Figure 8. ROM restriction after ACLR in the supine position. No restriction was observed in the near-isometric group (a) and restriction was observed in the non-isometric group (b, 35° restriction in this case).

Table 3

| Laxity (stable/slack/total) | Near-Isometric | Non-Isometric | P value |
|----------------------------|----------------|---------------|---------|
| 4th week                   | 11/1/12        | 8/4/12        | 0.316   |
| 8th week                   | 10/2/12        | 3/9/12        | 0.012   |
X-ray (Fig. 1b) and the anatomical specimen (Fig. 1c). A 1 mm k-wire with a blunt-ended probe was made to measure the length of the intercondylar line (Blumensaat’s line of rabbit) and the 60% point was marked to place the position of the non-anatomical femoral tunnel (Fe–N). The probe was placed along the intercondylar line (Fig. 1d) and the non-anatomical femoral tunnel was drilled with a 1 mm k-wire underneath the mark. The same approach was used with a 1 mm k-wire and a traditional anatomical femoral tunnel (Fe-A) was made through the center of the femoral footprint (Fig. 2a). The anterior (Ti-An), middle (Ti-Mi) and posterior (Ti–Po) tibial tunnel was made with 1 mm k-wire (Fig. 2b).

2.2. Measurement of length changes

The following creation of tunnels and the measurement of length changes in combined tunnel positions was conducted as previously reported by Smith [13] et al. Each time, two 2-0 MERSILK® (Johnson & Johnson co.) sutures were passed through the same tibial tunnel and along with the two femoral tunnels without suture twist. To measure the length change of the intra-articular point to point distance during knee flexion, a triple common node was made at the femoral cortex side of the suture to fix one end of the suture. Another suture nodule was made on the distal side approximately 10 mm to the tibial cortex and so the length change from the tibial cortex to the distal nodule was inverse to the intra-articular point to point length change (Fig. 3a). The increase (+) or decrease (−) in the intra-articular point-to-point distance were marked.

To ensure consistency of each measurement, a transparent flat baffle was placed to the right beside the thigh and the hind limb to keep the knee in the same coronal plane. The knee flexion angle was measured through the transparent baffle with the foot position marked at angles of 135°, 90°, 45° and 0°. Under the same position of the knee flexion movement, a constant tension (25 g weight) vertical to the tibial cortex was applied to the free end of the suture (Fig. 3b). The measurement starting point was set at a flexion angle of 135° in which the intra-articular point-to-point distance was also measured. After the initial lengths were measured, the patellar was restored from dislocation. The initial length of the paired point-to-point distances and the length change during the knee flexion at angles of 90°, 45° and 0° were obtained using an electronic Vernier caliper with an accuracy of 0.01 mm (Fig. 3c). To avoid investigator error, measurements were performed by two people and each measurement was repeated at least 3 times at every knee flexion angle and the average

![Figure 9.](Image)

**Table 4**

Macroscopic observation of tissue integration in intraarticular section.

| Time/Tissue integration | near-isometric | non-isometric | p Value |
|------------------------|---------------|---------------|---------|
| 4W                     |               |               | P<0.001 |
| Synovial tissue coverage| 6             | 1             |         |
| No tissue coverage      | 6             | 5             |         |
| Graft impair            | 0             | 6             |         |
| 8W                     |               |               | P<0.001 |
| Synovial tissue coverage| 8             | 2             |         |
| No tissue coverage      | 4             | 2             |         |
| Graft impair            | 0             | 8             |         |
value was recorded. The other measurements were performed as described above for the left two tibial tunnels combined with the femoral tunnels.

2.3. Animal experiment procedures

After the tunnel position isometry study, a total of 48 New Zealand white rabbits were randomly divided into two groups; one group with a near-isometric tunnel position and a non-isometric tunnel position group. A general ACLR procedure was built according to a previous study [22]. The ACL was visualized and dissected right from the enthesis. The knee was then flexed and a tibial tunnel with a 2.5 mm diameter was drilled within the ACL insertion area, anteriorly or posteriorly, using a Kirschner wire. A femoral tunnel was prepared at the center of the insertion site. An artificial PET ligament with a diameter of 2.5 mm was introduced through the bone tunnels by passing a suture that was fixed with a titanium interface screw (diameter 2.5 mm; length 6 mm) at the femoral site. PET ligament isometry (length change) was measured using the same method previously described with a constant tension of 5N. At the tibial site, the graft was fixed at a knee flexion of 30°/C14 with a constant graft tension of 5 N. A 3.0 mm diameter screw was used when the fixation with a 2.5 mm screw was not adequate. After the surgery, the range of motion in the knee was measured and the degree of restriction was recorded. Penicillin was given to the rabbits twice following the surgery. The rabbits were cage cared without immobilization. They had a full ROM and were checked twice a week to avoid joint adhesion. The rabbits were sacrificed at 4 and 8 weeks after the surgery. The anteroposterior laxity of the knee was compared to the contralateral intact knee by two researchers at a knee flexion of 90° after sacrifice. The normal knee anteroposterior laxity of the rabbit at a flexion 90° in the supine position was almost 0 in the anteroposterior tibial movement. The laxity of the knee was slack or stable and was independent of the anteroposterior movement.

2.4. Macroscopic observations

After sacrifice, the graft integration of the intra-articular section was recorded with the assessment of synovial tissue coverage and graft impairment by macroscopic observations.

2.5. Micro-CT analysis

Each of the freshly harvested femoral–graft–tibia complex samples was scanned in a SkyScan 1176 micro-CT (Bruker Co. Ltd, Billerica, Massachusetts, USA) at an energy of 80 kV and a current of 278 μA with a 0.5 mm aluminum filter. The scans were performed using a 9 μm pixel size with a 0.5° rotation step and 180° total scan rotation. The images were reconstructed and analyzed by the scanner software NRecon, Data Viewer and CT An (Bruker Co. Ltd.) as previously described [23]. A column (3.0 mm in diameter and 1.5 mm in height) within the trabecular region centered on the longitudinal axis of the PET graft was defined as the region of interest (ROI-3D). The bone volume per total volume (BV/TV) and the cross-sectional area of the bone tunnel at the joint surface were detected and analyzed.

2.6. Histological assessment

After micro-CT scanning, the samples were decalcified in Plank-Rychlo decalcifying fluid for 2 weeks at room temperature. After dehydrating the samples in a graded series of ethanol and embedding in paraffin, the samples were cut into 4 μm sections perpendicular to the longitudinal axis of the graft using a microtome (SM2500; Leica, Nussloch, Germany). The sections were stained with hematoxylin-eosin and Masson’s trichrome and were observed using an inverted light

Figure 10. Micro-CT results of the tibial tunnel area (a1, b1, c1, d1) and BV/TV at the joint side in the 3D reconstruction (a2, b2, c2, d2) with quantification (e, f). Arrows: bony ingrowth to graft. Red circle: original tunnel. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
microscope (IX71SBF-2, Olympus Optical Co., Tokyo, Japan). Digital images were acquired using a DP Manager (Olympus Optical Co.).

2.7. Biomechanical tests

Routine procedures for the biomechanical tests were followed as previously described [24] using an electronic universal materials testing system machine (AGS-X, Shimadzu, Kyoto, Japan). The femur–graft–tibia complex without fixation removal was prepared. The testing was carried out with an elongation phase of 6 mm/min. The ultimate failure loads were recorded for the 4 specimens in each group at each follow-up time. Additional biomechanical tests were performed on the 4 native intact knees and on the 4 time zero reconstruction complexes.

2.8. Statistical analysis

The percentage change in length was obtained from the original data. Comparison of the distance changes in different tunnel positions was performed using a one-way analysis of variance with a post hoc Tukey multiple test. Differences in the two group variables were analyzed using an unpaired Student’s t-test, analysis of variance, and a Mann-Whitney’s U test as appropriate. Data analysis was performed using GraphPad Prism8 (GraphPad Software, San Diego, CA) and a p-value threshold of <0.05(*) was considered statistically significant. A p-value of <0.001(***) was considered highly significant.

3. Results

3.1. Patterns of tunnel position length changes

The minimum initial length of the tunnel position was the femoral anatomical position paired with the tibial posterior position 9.10 (±0.92) mm. The maximum length was the femoral non-anatomical position paired with the tibial anterior position 12.49 (±1.11) mm (Table 1). The initial length increased as the tibial tunnel moved anteriorly. During knee motion between 135° and 10°, the point to point distance reach a maximum when the rabbit knee flexion was 135°, and decreased as the knee was extended (Table 1, Fig. 4). The minimum length change combinations were the femoral anatomical position paired with the tibial posterior position 0.48 (±0.18) mm which was 5.19 (±1.78) % to the initial length. The largest length change position was at the femoral non-anatomical and the tibial anterior position −2.72 (±0.43) mm which was

Figure 11. Synovial tissue coverage and ingrowth to the PET graft of the intra-articular section (a, b, c, d) with quantification of the width of tissue coverage (e). Yellow Arrows: coverage of synovial graft. Green arrows: PET fibers; *: ingrowth; Mark bar: 100um. Near-I: near-isometric. Non-I: non-isometric. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
21.94 (±2.24) % to the initial length (Fig. 5). The center of the anatomical position (femoral anatomical paired with the tibial middle) resulted in a distance change of 0.98 (±0.30) mm which was 9.36 (±2.56)% of the initial length. In the least length change pair of the tunnel position cases, only one case resulted in a close to isometric change with 1.36% of the maximum length change during knee motion of 135 – 10°. In contrast to the pattern of decreasing length change with knee extension, 1 of the smallest length change pair of tunnel position cases resulted in an increasing and then decreasing trend and 2 resulted in a decreasing/increasing trend during knee extension. There were no exceptions in the other 51 cases.

3.2. Maximum length changed during knee motion from 135° to 45°

Considering rabbit daily behaviors and knee activity, the maximum length change difference of the six paired positions were compared under knee flexion angles from 135° to 45°. Three significantly different tunnel position combinations were observed that resulted in length changes (P<0.001). These were combinations of the femoral anatomical and tibial posterior position (−1.73 ± 0.34 mm, p<0.001), the femoral anatomical and tibial anterior position (−0.22 ± 0.22 mm) and the femoral non-anatomical and tibial anterior position (−0.97, ±0.30mm). The percentage of length changes in these positions were −2.45(±2.16)%, −8.43(±2.43)%, −13.89(±2.19)% respectively (P<0.001) (Fig. 6). While the other tunnel positions, the statistical differences were not significant (P<0.05). Thus, the near-isometric tunnel position was Fe-A and Ti-Po with minimal isometry. The combination of Fe-A and Ti-An was selected as the non-isometric tunnel position which significantly different from the minimal isometry combination.

3.3. Femoral versus tibial influences on tunnel position length changes

To compare the influence of tunnel positions between the femoral and tibial selections, the changed length values between the two tunnel positions from one side (femoral or tibial) paired with the same tunnel position from the other side (tibial or femoral) were calculated (Fig. 7). The changes in length between the femoral anatomical and the non-anatomical tunnel positions (Fe△N-A) paired with the same tibial tunnel positions were largest compared to the values in the tibial tunnel positions (P<0.01). These data suggest that the femoral tunnel has a greater influence on the length changes. As the pattern of length change increased with the anterior tibial tunnel selection, the lengths changed from the tibial posterior position to the tibial middle (Ti△mi-po) and tibial anterior (Ti△an-po) position increased.

3.4. Graft isometry, ROM restriction and knee laxity in the animal experimental model

The graft isometry of the near-isometric and non-isometric groups in

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**Figure 12.** The graft-bone interface of the tibial tunnel by H&E (a1, b1, c1, d1) and Masson’s staining (a2, b2, c2, d2) at 4 and 8 weeks and quantification (e: femoral; f: tibial). The graft-bone interface is shown by the orange line. Arrow in C1: bone ingrowth to graft. Mark bar: 100 um. Near-I: near-isometric. Non-I: non-isometric. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Figure 13.** The maximum failure load of the intact ACL at each follow-up time. In: intact. T0: time zero. Near-I: near-isometric. Non-I: non-isometric.
the animal experiments after ACLR were 0.54 (±0.28) mm and 1.63 (±0.29) mm, respectively (p<0.0001). There was no ROM restriction in the near-isometric group (0/24) after surgery and at follow-up. In the non-isometric group, the ROM restriction occurred 22.50 (±14.14) in most cases after surgery (23/24) (Fig. 8). On the 4th week, there were 3 ROM restrictions 8.75 (±6.29) remaining and no ROM restrictions were observed (0/12) at the 8th week after surgery (Table 2). As there was ROM restriction, the knee laxity at a flexion angle of 90° in the supine position was normal after surgery, however, at the 8th week of follow-up, the levels of knee laxity slack and were higher in the non-isometric group (9/12) compared to the near-isometric group (2/12) (Table 3).

3.5. Macroscopic observations

As shown in Fig. 9, synovial tissue coverage was observed in the near-isometric group at both follow-up times. However, no tissue coverage or graft impairment was observed in the non-isometric group. A significant difference (p<.001) was found in the two groups (Table 4).

3.6. Micro-CT analysis

The tibial bone tunnels were analyzed by measuring the average tunnel area and the BV/TV value at 4 and 8 weeks after surgery as shown in Fig. 10. The tunnel area was smaller in the near-isometric group at both 4 and 8 weeks (5.804 ± 0.4173 mm² VS 6.855 ± 1.238 mm² p = 0.037, and 6.069 ± 0.6967 mm² VS 7.510 ± 1.102 mm² p = 0.007). Compared to the original tunnel area (6.25 mm²), the mean values were smaller in the near-isometric group and larger in the non-isometric group at both follow-up times. The BV/TV values were higher in the near-isometric group compared to the non-isometric group both at 4 and 8 weeks (11.250 ± 0.4037 VS 5.735 ± 2.761%, p = 0.013, and 15.820 ± 3.424% VS 10.730 ± 3.740%, p = 0.007).

3.7. Histological assessment

Histological analysis was conducted at the graft in the bone tunnel and the intra-articular section. Based on H&E staining, a distinct synovial tissue coverage was noticed in the near-isometric group at 4 and 8 weeks with quantitative differences (p<0.05). Synovial ingrowth was also observed in both groups as shown in Fig. 11. Bone-in growth was observed in the near-isometric group at 8 weeks (Fig. 12, c1, yellow arrow). However, the widths of the interface between the bone and the graft at 4 and 8 weeks were not statistically different as shown in Fig. 12.

3.8. Biomechanical tests

All specimens failed by pullout from the tibial bone tunnels and no graft rupture occurred. No significant differences were observed between the two groups at each follow-up time after surgery (10.4 ± 29.05N vs. 109.2 ± 21.49N at the 4th week, 156.8N ± 25.98N vs. 114.6 ± 26.04N at the 8th week). The failure loads in the near-isometric group at 8 weeks were significantly higher than at time-zero reconstruction (156.8N ± 25.98N vs. 102.6 ± 22.96N p = 0.02) which were approximately 84% of the mean intact ACL ligament failure load (187.0 ± 26.40N) (Fig. 13).

4. Discussion

Isometric ACLR means that the distance between the femoral and tibial attachments of the reconstruction remains constant as the knee is moved in flexion/extension [21]. Such placement may prevent the graft from becoming excessively tense in extension and thereby constraining joint motion or increased anterior translation resulting in slackening of the graft as the knee is flexed and extended [7]. It is difficult to locate the absolute isometric tunnel position in surgery because of individual differences including a lack of bony structure and surgical experience, therefore, surgeons prefer isometric reconstruction in ACLR [30]. However, the acceptable isometry of the graft remains controversial and clinical studies[31-33] have shown no advantage in certain tunnel positions with clinical outcomes or graft maturity. An animal model study may provide some evidence to support the advantages of near-isometric reconstruction. Limited studies have focused on tunnel position isometry when performing ACLR in an animal model. It is meaningful to establish a preclinical animal model of reproducible near-isometric ACLR.

In this study, we developed a rabbit model as different tunnel positions could be placed under direct viewing that was reproducible. Also, the isometry of the tunnel position combinations and knee laxity could be manually measurable in rabbits. The minimal size of the PET ligament and fixation screw set were also adaptable to the rabbit model which play key roles in achieving optimum biomechanics. As a result, near and non-isometric tunnel position combinations were found both in the anatomical region of the footprint. The tunnel combination with minimal isometry (near-isometric) was the Fe-A paired with Ti-Po and the worst combination was Fe-N and Ti-an.

The percentage length changes in the tunnel positions were recorded during knee motion from the initial knee flexion angle which was 135°. Our results showed this was effective and necessary and resulted from the outcome between the tunnel combinations of the Fe-A paired with Ti-an and the Fe-N paired with Ti-po. Similar length changes were observed but the initial lengths were different. The percentage length changes reflected the graft-tunnel strain during the range of knee motion. A more posterior tibial tunnel position had better isometry and a more femoral tunnel position had a larger effect on isometry. The tunnel position (Fe-A and Ti-Po isometry 2.45 ± 2.16%) with minimal isometry was chosen as the near-isometric group. Another tunnel position that was significantly different (Fe-A and Ti-an, isometry 8.43 ± 2.43%) was used as the non-isometric group, both in anatomical footprint.

In the present study, we preliminary studied the short-term effects on PET graft-bone integration between the near-isometric ACLR and the non-isometric ACLR. The knee ROM restriction occurs in some patients [28] with artificial ligaments in clinical practice. The ACLR flexion angle in the present study was set to 30° where the tunnel position length was short. This allowed us to observe the relationship between isometry and ROM restriction. Effective ROM restriction was observed in the non-isometric group. However, no ROM restriction occurred in the near-isometric group under the same fixation method. Integration of the intra-articular section was higher in the near-isometric group based on macroscopic observations and histological analysis. Micro-CT also showed better bone integration and less tunnel enlargement in the near-isometric group. Non-isometric ACLR led to ROM restriction that increased graft-bone strain and resulted in tunnel enlargement and graft impairment.

A previous study focused on the impact of high graft strain on graft-bone integration [20]. In this study, a relatively high force of the initial graft strain was made to observe the difference in ROM restrictions between the near-isometric and non-isometric tunnel positions. And notably, an additional outcome of knee stability change was observed in the present study. A restricted ROM may improve stability after ACLR, however, the ROM restriction decreased at week 4 and disappeared at week 8 whilst the knee laxity was worse in the non-isometric group (9/12) at 8 weeks of follow-up. These observations may be due to high graft-bone strain that led to graft impairment and tunnel enlargement. The ideal fixation strategy with a non-isometric tunnel position is fixation at a flexion angle where the tunnel position distance is largest to avoid tunnel enlargement and graft impairment. Stability changes were also observed in the near-isometric group (2/12) on week 8. These observations may be because the distance of the tunnel position was longest at full knee flexion in the rabbit model. This caused high graft-tunnel stress at full knee flexion. Based on tunnel position isometry, the graft in the rabbit model of ACLR is exposed to a relatively high load in the post-operative daily rest state.

Biomechanical tests were conducted without removing the fixation screw. The maximum failure load was superior to previous articles.
The near-isometric group achieved the maximum failure load at the 8th week and was 84% of the mean intact ACL ligament. The maximum failure load was significantly higher than the time zero reconstruction. PET ligaments require a firm fixation method and the integration of fixation–graft–tunnel complex would be concerned in biomechanical evaluation.

This study has several limitations. To evaluate the effectiveness of different isometry of tunnel positions, we designed a fixation angle with relatively high strain and used an artificial ligament that was more sensitive to tunnel isometry than an autograft. More studies with a variable graft and an ideal fixation strategy are needed in this animal model. Graft-tunnel interface width did not result in statistical difference, rather than result shown in micro-CT analysis. This might due to the small sample size and the histology tissue slice was unable to be as accurate as micro-CT analysis. The anteroposterior laxity of the knee was not quantitatively measured, however, the anteroposterior tibial movement was estimated by manual examination. In the normal knee at a flexion angle of 90°, the anteroposterior tibial movement was zero in the supine position. A customized device for quantitatively measuring the laxity of the knee needed in future studies.

In conclusion, we established a rabbit model of ACLR tunnel positions with differential isometry containing near-isometric and non-isometric positions. The near-isometric tunnel position was located at the anatomical center of the femoral position combined with the posterior tibial position. This model enable further research into ACL surgical strategies using variable grafts, graft healing, graft-bone integration and rehabilitation based on tunnel position isometry.

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Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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