A Time-Zero Biomechanical Study of the Effective Length of the Graft in Posterior Cruciate Ligament Reconstruction

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Abstract

Background The effect of effective length on the biomechanical properties of the graft is regarded as an essential variable influencing the posterior cruciate ligament reconstruction. However, the effect has not been fully studied. The purpose was to compare the effects of different effective graft lengths (35 mm, 55 mm, 65 mm) on the time-zero biomechanical properties of the graft in posterior cruciate ligament (PCL) reconstruction.

Methods Bovine digital flexor tendons and porcine tibias were used to establish in-vitro PCL reconstruction models. Tensile strength testing was performed at 3 different effective lengths of the graft: short (35 mm, n = 10, group 1), medium (55 mm, n = 10, group 2), and long (65 mm, n = 10, group 3). A computer software (Trapezium X; Shimadzu) was used to record the load-elongation curve, ultimate load (N), the elongation of the graft during the test (mm), tensile stiffness (N/mm), and energy absorption (J). The failure pattern was evaluated by visual observation.

Results All the samples failed because the grafts slipped out from the bones, except two grafts ruptured in group 1. The tensile stiffness and ultimate load in group 1 were significantly higher than those in group 2 and group 3 (tensile stiffness, 50.49 ± 11.43 N/mm in group 1 vs 31.20 ± 10.44 N/mm in group 2 [P < 0.001] and 19.18 ± 6.18 N/mm in group 3 [P < 0.001]; ultimate load, 452.40 ± 54.52 N in group 1 vs 338.50 ± 26.79 N in group 2 [P < 0.001] and 268.70 ± 28.30 N in group 3 [P < 0.001]). There were significant differences between group 1 and group 3 in energy absorption (9.61 ± 3.25 J vs 5.22 ± 2.43 J, P = 0.002). At 50 N and 100 N of applied load, no statistically significant differences were detected on the elongation of the grafts (P > 0.05). The elongation of the short grafts under 150 N and 200 N of applied load was significantly less than that of the long grafts (150 N, 1.77 ± 0.83 mm in group 1 vs 4.14 ± 1.75 mm in group 3, P = 0.047; 200 N, 2.62 ± 1.10 mm in group 1 vs 7.06 ± 3.20 mm in group 3, P = 0.006).

Conclusions This study demonstrated the time-zero biomechanical properties of the graft with short effective length were superior to those of the graft with medium and long effective lengths in PCL reconstruction.

Background

Posterior cruciate ligament (PCL) injury is a common type of acute knee injuries, accounting for 1% to over 40% [1–4]. This injury is an important cause of knee joint kinematic diseases such as joint hyperextension and joint instability, which is mainly caused by sporting activities (38.8%) and traffic accidents (35%) [5, 6]. Abnormal knee kinematics was considered to be the reason of increased cartilage degeneration of the knee [7, 8]. A long-term follow-up study showed that the knee joint might lose function after conservative treatment [9]. Therefore, a clear diagnosis and appropriate treatment strategy for PCL injury are very important. With the development of surgical techniques and the improvement of clinical therapeutic results, PCL reconstruction is becoming more common, and autologous or allogeneic graft reconstruction is currently the main treatments for such injuries[10].
The purpose of posterior cruciate ligament reconstruction is similar to that of anterior cruciate ligament (ACL) reconstruction, both of which are to restore optimally the kinematic properties of the normal knee and prevent the occurrence of osteoarthritis in the later stage. Accordingly, the reconstructed graft should reproduce the biomechanical properties and function of the original intact ligament as far as possible. Many studies reported that there are a lot of factors affecting the effect of PCL reconstruction, such as graft type, graft size, bone tunnel placement, fixation technique and postoperative rehabilitation [11–16]. In evaluating the factors affecting the effect of PCL reconstruction, graft stiffness and in-situ force are two key biomechanical property indicators. DeFrate et al. [17] investigated the effective PCL graft length which directly affected the biomechanical properties of the ligament after reconstruction through a cadaveric study. They studied 3 different effective lengths (represented 3 kinds of fixation techniques) by using Achilles tendon graft, and showed that the short grafts had largest stiffness and least overall elongation than the medium grafts and the long grafts. However, this study did not consider some factors that may affect the biomechanical properties of the graft in an actual bone tunnel, such as graft-tunnel wall interactions, the material of the fixing device, and the way of fixation used. Li et al. [18] established a piecewise non-linear function model to study the force-elongation behavior of the PCL grafts, meanwhile, a minimal deformation energy method was used to analyze the biomechanical properties of the graft with different effective length. Their analyses demonstrated that there is a specific optimal length of the different graft material to simulate the PCL and the shorter graft had higher graft structural resistance than the longer one. Nevertheless, this study was only a theoretical analysis and the actual effect had not been verified when using the theories of this study to explain PCL reconstruction. According to our retrieve, there were few studies have reported the time-zero biomechanical properties of the graft with different effective length in PCL reconstruction. Therefore, this study investigated the time-zero biomechanical properties of the graft with different effective length in PCL reconstruction. We hypothesized that the short effective graft length would have superior biomechanical properties.

**Materials And Methods**

**Preparation of PCL reconstruction models**

Our study was performed using 30 bovine digital flexor tendons and 30 fresh-frozen porcine tibias from pigs aged 10–14 months. To simulate the model of transtibial PCL reconstruction more conveniently, we removed all soft tissues, including skin, subcutaneous tissue, ligaments (tibial footprint of the PCL insertion was reserved), and muscles from the tibia. Many previous studies have demonstrated that porcine tibias and bovine digital flexor tendons were similar to human tibias and ligaments in biomechanical, structural, and material properties [19–22]. Each group of specimens was wrapped in 0.9% saline-soaked gauze, placed in a resealable specimen bag, and stored at -20°C until required for testing[20, 21].

Before testing, the tendons and tibias were thawed overnight at 4°C. We dissected and separated all bovine digital flexor tendons to obtain eligible grafts. Then, the grafts were folded once to make a double loop (length: 150 mm; diameter: 8 mm). The ends of the grafts (approximately 50 mm long) were sewn
using #2 Ethibond non-absorbable suture (Ethicon, Somerville, NJ) with whipstitch. Referring to previous studies, we adjusted the graft to match the diameter of the tunnel, and appropriate resistance should be felt after placing the graft into the tibia tunnel[23, 24]. The length of the graft was consisted two parts, the one part was approximately 50 mm intraosseous length (tibia tunnel length) and the other part was approximately 100 mm extraosseous length (the length from the outlet of the tibial tunnel to the clamp and the remaining length for fixation by the clamp). In order to minimize viscoelasticity, all grafts underwent preconditioning (approximately 20 N for 15 minutes) before each test. When we processed the grafts, all samples were wrapped in gauze soaked in 0.9% sterile saline until test.

The tibias were cut distally perpendicular to the tibial bone shaft at 15 cm roughly from the tibial plateau. According to previous studies [25, 26], the transtibial tunnel technique was used to create the tibial tunnel, and a tibia drill guide system (Arthrex Inc, Florida, USA) was used. We established the tibial tunnel by the anteromedial portal, and the portal onto the tibial anteromedial cortex was 4.5 cm below the tibial plateau and 1.5 cm medial to the tibial tuberosity. The headend of the system was located in the lateral portion of the PCL anatomic tibial footprint. To fit the size of the porcine bone, the orientation of the drill guide system was adjusted and fixed at 70°. A guide pin was inserted into the slightly lateral portion of the PCL tibial attachment site, approximately 1 cm distal to the articular surface. A cannulated reamer (8-mm, the same diameter as the prepared graft) was drilled from the anteromedial cortex to the lateral portion PCL tibial attachment site to create the tibial tunnel (length: 50 mm; diameter: 8 mm). We paid our special attention to protecting the tibial tunnel from split fracture and clearing the bone debris in the tunnel in each group.

Study Groups And Fixation Techniques

According to previous studies, we designed 3 different effective graft lengths to investigate the effective length on the biomechanical properties of the graft[17, 18, 27–29]: short (35 mm, n = 10, group 1), medium (55 mm, n = 10, group 2), and long (65 mm, n = 10, group 3). The 3 groups all used distal tibial fixation in transtibial techniques. The grafts in different groups had roughly the same length in the bone tunnel (approximately 50 mm), but the extraosseous length was different (Fig. 1). The short, medium, and long grafts represented proximal fixation within the femur tunnel (near the articular surface, 35 mm), midtunnel fixation on the femur (55 mm), and distal fixation on the femur (65 mm), respectively. A single titanium interference screw (8 x 25 mm, ConMed Linvatec, Florida, USA) was inserted between the tibial tunnel and graft to fix the graft, and we aligned the screw tail with the entrance of the tibial tunnel.

Mechanical Testing

We fixed the tibia in a steel sleeve, and clamped the graft tightly with a testing jig. A kirschner wire was located 2 cm below the tunnel entrance and crosses the tibia to fix the tibial[24]. In order to simulate a normal PCL (anterior declination approximately 70°~80°)[30], we set the angle between the orientation of the bone tunnel and the tensile force on the sagittal plane at about 130° (we used a saline spray to make grafts moist during testing). A universal material testing machine (Shimadzu Inc, Kyoto, Japan) was used for tensile strength testing and a computer software (Trapezium X; Shimadzu) was used to collect and
process all data. Ultimate tensile strength of every sample was measured by pull-out testing at 10 mm/min and the testing completed when the graft pulled out of the tunnel. The computer software recorded the load-elongation curve, ultimate load (N), the elongation of the graft during the test (mm), tensile stiffness (N/mm, the slope of the linear region of the load-elongation curve), energy absorption (J, the maximum efficiency of the testing machine during the test). Failure pattern was evaluated by visual observation.

Statistical analysis

We referred to the biomechanical data from earlier experiments and determined the necessary experiment sample size, altogether 30 in-vitro PCL reconstruction models were used in this study[20, 31, 32]. The results were analyzed by SPSS software (version 22.0; IBM, Armonk, NY, USA). The mean differences between 3 groups were used one-way analysis of variance (ANOVA), and p-value < 0.05 was defined statistical significance.

Results

Basic characteristics of experimental samples

We found there were no significant differences in the basic characteristics of the grafts and tibias (Table 1). The results of the graft pre-tensioning, tibia tunnel length, and tibia tunnel angle were presented as mean ± standard deviation (SD), and no significant differences were found among the groups (P > 0.05).

| Characteristic     | Group 1 (n = 10) | Group 2 (n = 10) | Group 3 (n = 10) | Group 1 vs Group 2 | Group 1 vs Group 3 | Group 2 vs Group 3 |
|-------------------|-----------------|-----------------|-----------------|-------------------|-------------------|-------------------|
| Pre-tension, N    | 20.73 ± 1.18    | 20.49 ± 1.33    | 20.93 ± 1.27    | .674              | .726              | .442              |
| Tunnel length, mm | 51.01 ± 1.52    | 51.31 ± 1.35    | 50.77 ± 1.10    | .619              | .691              | .374              |
| Tunnel angle, °   | 69.70 ± 0.59    | 69.68 ± 0.72    | 69.57 ± 0.84    | .951              | .690              | .736              |

Results Of Mechanical Testing
There were no significant differences in failure patterns. All the samples failed because the grafts slipped out from the bones, except two grafts in group 1 were ruptured. We showed representative load-elongation curve in each group (Fig. 2).

ANOVA showed significant differences in the tensile stiffness, ultimate load between the 3 groups (P < 0.05) (Table 2). The tensile stiffness, ultimate load in group 1 were significantly greater than those in group 2 and group 3 (tensile stiffness, 50.49 ± 11.43 N/mm in group 1 vs 31.20 ± 10.44 N/mm in group 2 [P < 0.001] and 19.18 ± 6.18 N/mm in group 3 [P < 0.001]; ultimate load, 452.40 ± 54.52 N in group 1 vs 338.50 ± 26.79 N in group 2 [P < 0.001] and 268.70 ± 28.30 N in group 3 [P < 0.001]). Among the 3 groups, there was only a significant difference in energy absorption between group 1 and group 3 (9.61 ± 3.25 J in group 1 vs 7.25 ± 3.05 J in group 2 [P = 0.081], and 5.22 ± 2.43 J in group 3 [P = 0.002], 7.25 ± 3.05 J in group 2 vs 5.22 ± 2.43 J in group 3 [P = 0.130]).

### Table 2

Biomechanical Properties of Specimens

| Biomechanical Properties | Mean ± Standard Deviation | P Value |
|--------------------------|--------------------------|---------|
|                          | Group 1 (n = 10)         | Group 2 (n = 10) | Group 3 (n = 10) | Group 1 vs Group 2 | Group 1 vs Group 3 | Group 2 vs Group 3 |
| the elongation at 50N, mm | 0.41 ± 0.19              | 0.75 ± 0.81      | 0.93 ± 0.70      | 0.231              | 0.072              | 0.526              |
| the elongation at 100N, mm | 1.01 ± 0.50              | 2.12 ± 3.38      | 2.22 ± 1.22      | 0.244              | 0.207              | 0.920              |
| the elongation at 150N, mm | 1.77 ± 0.83              | 3.39 ± 3.96      | 4.14 ± 1.75      | 0.166              | 0.047              | 0.519              |
| the elongation at 200N, mm | 2.62 ± 1.10              | 4.88 ± 4.61      | 7.06 ± 3.20      | 0.138              | 0.006              | 0.151              |
| Tensile stiffness, N/mm  | 50.49 ± 11.43            | 31.20 ± 10.44    | 19.18 ± 6.18     | 0.000              | 0.000              | 0.009              |
| Ultimate load, N         | 452.40 ± 54.52           | 338.50 ± 26.79   | 268.70 ± 28.30   | 0.000              | 0.000              | 0.000              |
| Energy absorption, J     | 9.61 ± 3.25              | 7.25 ± 3.05      | 5.22 ± 2.34      | 0.081              | 0.002              | 0.130              |

Regarding the elongation of grafts with different effective lengths under different applied loads, we recorded the elongation of the graft under 50 N, 100 N, 150 N and 200 N of load. At 50 N and 100 N of applied load, the elongation of grafts with different effective lengths was not statistically significant (P > 0.05). The elongation of the long grafts was statistically greater than that of the short grafts at 150 N of load (1.77 ± 0.83 mm in group 1 vs 4.14 ± 1.75 mm in group 3, P = 0.047). At 200 N of applied load, the
results also showed significant differences among the group 1 and group 3 (2.62 ± 1.10 mm in group 1 vs 7.06 ± 3.20 mm in group 3, P = 0.006).

Discussion

The principal findings of this study were that the graft with short effective length exhibited superior time-zero biomechanical properties to the graft with medium and long effective length in the aspect of tensile stiffness and ultimate load in PCL reconstruction. In addition, the elongation of the short grafts under 150 N and 200 N of applied load was significantly less than that of the long grafts.

In our study, the current results supported our initial hypothesis and were consistent with the conclusions of previous biomechanical study. DeFrate et al. [17] carried out a biomechanical study through Achilles tendon grafts and demonstrated that the linear stiffness and graft resistance of the graft would be increased statistically by decreasing the effective length of the graft from long to short. In contrast, the conclusions of our study were more in line with actual PCL reconstruction because we considered the effects of graft-tunnel wall interactions and fixation techniques in actual bone tunnel.

The purpose of PCL surgical reconstruction is to reproduce the biomechanical properties of PCL. In order to achieve this purpose, the graft should match the intact PCL in biomechanical properties. However, there is still no consensus on many factors affecting the reconstructive outcomes [1, 33–37]. According to the findings of our study, the effective length of the graft should be considered as an important factor influencing the reconstructive outcomes in PCL reconstruction. A theoretical analysis studied the effect of effective graft length on the biomechanical properties of the graft in PCL reconstruction: Li et al. [18] established a piecewise non-linear function model and used a minimal deformation energy method to analyze the effect of effective graft length on the biomechanical properties of the PCL graft of different materials. Their analyses demonstrated that there is a specific optimal length of the different graft material to simulate the PCL and the shorter graft had higher graft structural resistance than the longer one. Therefore, the effective length of the graft should be adjusted according to different graft materials and shortened as much as possible within the appropriate range.

According to a biomechanical engineering theory, the length of a graft is an important factor to determine the biomechanical properties of the graft [18]. In our study, the effective length of the graft increased with the extraosseous length. This led to decreased graft stiffness and increased graft deformation [17, 38]. Hence, a reasonable interpretation for the improvement in biomechanical properties of the short effective graft length group was that it had greater stiffness. Zhang et al [39] performed a biomechanical study to measure the graft stiffness of different fixation methods (distal, combined, and proximal fixation methods) in tibia tunnel. They reported that the proximal fixation group showed greater stiffness compared with distal group because the graft with a shorter effective length in the proximal group.

Another study on ACL reconstruction also agreed well with our viewpoint: Ishibashi et al. [38] assessed the effects of 3 different fixation sites (distal, central, and proximal fixation methods) on the biomechanical properties of the graft in ACL reconstruction. They found that the most stable knee was
produced by proximal fixation (The graft with a short effective length) and this fixation technique produced superior results regarding the in-situ forces of the graft, anterior displacement of the tibia, and internal rotation of the tibia compared with distal and central fixation.

Regarding the ultimate load, a previous study on PCL reconstruction suggested that the force of PCL graft should reach approximately 350 N in vivo load in normal walking [40]. Ahn et al. [25] build 2 groups in-vitro PCL reconstruction models with different approaches (10 anteromedial and 10 anterolateral approaches) in a transtibial PCL reconstruction technique by using cadaveric tibias. They measured the maximum load of the graft at failure after the Achilles tendon allograft was fixed with a biodegradable screw, and found that the anteromedial group was 385.4 ± 139.7 N and anterolateral group was 225.1 ± 144.1 N. Lee et al. [24] performed a biomechanical study and used a bio-interference screw to perform tibial fixation of the graft, they found that the mean ultimate load was 371.3 ± 106.2 N. The mean ultimate loads in our study in groups 1, 2, and 3 were 452.40 ± 54.52 N, 338.50 ± 26.79 N, 268.70 ± 28.30 N, respectively. We found the mean ultimate load of group 1 was higher than that of group 2 and group 3, and the difference was statistically significant among the 3 groups. The ultimate load of the graft decreased sharply as the graft effective length increased. A potential reason was that the graft with short effective length had greater stiffness which influenced its structural resistance directly. Hence, the conclusion of our study in that the PCL graft with short effective length had satisfactory mean ultimate load was convincing.

Regarding the elongations of grafts with different effective lengths under different load levels, our results indicated that the elongation of the graft with short effective length was less than that of the long one under 150N and 200N of applied loads. In our opinion, the reason was the graft with short effective length had greater graft resistance. DeFrate et al. [17] found that the differences in elongation between the graft with different effective lengths at lower loads were relatively small. Nevertheless, with increasing applied loads, the elongation of the graft with short effective length was statistically less than that of the long one. Hence, their results were consistent with our study. DeFrate et al. considered the graft will transmit same force with the intact PCL at low applied loads, however, at higher applied loads, the graft with long effective length cannot reproduce the biomechanical properties of the intact PCL by reason that the non-linear force-elongation behavior of the graft. Therefore, this could draw a conclusion that a suitable graft effective length should be chosen to restore the biomechanical properties of the intact PCL in a large range of load.

Limitations

Our study still has certain limitations that must be acknowledged. First, we used the porcine tibia and bovine digital flexor tendon to simulate the human knee, which may not applicable in clinical surgery. However, in reference to previous studies, the consistency, accessibility and quality of porcine bone were similar to young human bone and the bovine digital flexor tendon is very close to the tendon of young human in structural and material properties [41, 42]. Furthermore, it is difficult to obtain human specimens, and factors such as the age of the donor are difficult to control. Second, in this study, only the
time-zero outcomes after PCL reconstruction were studied, and we did not carry out cyclic loading. Future studies could perform cyclic loading to simulate daily activities and explore tendon-bone healing on the PCL graft. Third, we selected a titanium interference screw which is not commonly used in PCL reconstruction surgery instead of a bio-interference screw. The main reason was the soft-tissue fixation properties of screws of different materials are similar [20]. In addition, many previous papers have used metal screw to fix graft in biomechanical studies [39, 43].

Conclusions

This study demonstrated the time-zero biomechanical properties of the graft with short effective length were superior to those of the graft with medium and long effective lengths in PCL reconstruction.

Abbreviations

ACL: Anterior cruciate ligament; PCL: posterior cruciate ligament; ANOVA: one-way analysis of variance; SD: standard deviation.

Declarations

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Availability of data and materials

The data and materials in this paper may be made available upon request through sending e-mail to first author.

Authors’ contributions

FT, YJT, FL, XHZ, HH and YYX designed the study; FT, YJT, FL, XHZ, ZCL and HW prepared the samples and performed the test; FT, YJT, HH, LZH and YYX analyzed and interpreted the data; FT, YJT, and XHZ wrote the initial draft; FT, YJT, HH, LZH, HW and YYX ensured the accuracy of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The protocols for collection, process, and testing of bovine digital flexor tendons and porcine tibias were reviewed and approved by the Ethics Committee on the Care and Use of Animals of the Lanzhou University Second Hospital, and all animals received humane care in strict accordance with the National Institutes of Health Guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 1

Effective length of the graft in transtibial posterior cruciate ligament reconstruction. The effective graft length of group 1 was 35 mm; group 2, 55 mm; and group 3, 65 mm.

Figure 2

Representative load-elongation curves in 3 groups. The ultimate load of each group was labeled.