

## Original Article

# Biomechanical properties of reinforced bone-anterior cruciate ligament-bone allografts for anterior cruciate ligament reconstruction in rabbits

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**Abstract:** Objective: The objective of present study was to investigate the initial biomechanical properties of a reinforced bone-anterior cruciate ligament-bone (RB-ACL-B) allograft in the reconstruction of the ACL. Method: Seventy-five mature New Zealand rabbits were used in this study. Thirty rabbits were used to prepare the B-ACL-B allograft, while the remaining rabbits were randomly divided into three groups (n = 15): the first group underwent ACL reconstruction with the B-ACL-B allograft (B-ACL-B group); the second group, with a 4-stranded semitendinosus tendon (ST) autograft (ST group); and the final group with a 2-stranded ST-reinforced B-ACL-B allograft (RB-ACL-B group). The non-operated contralateral leg served as the normal control (NC group). The biomechanical properties of the repair were tested in five rabbits from each group on 16, 32 and 48 weeks, postoperatively, to assess the efficacy of the RB-ACL-B allograft in ACL reconstruction. Result: The mean maximum force for the RB-ACL-B allograft was 41%, 58%, and 68% of NC group (P < 0.05) at 16, 32, and 48 weeks, respectively. Compared with mean maximum forces of 31%, 45%, and 55% for the ST group (P < 0.05) and 23%, 34%, and 38% for the B-ACL-B group (P < 0.05). Although the biomechanical properties of the experimental groups failed to reach the level of the NC group, the biomechanical properties of the RB-ACL-B group were better than those of the ST and B-ACL-B groups. Conclusion: The RB-ACL-B allograft has good biomechanical properties and offers superior outcomes over the ST and B-ACL-B grafts in ACL repair. The RB-ACL-B allograft may offer an effective solution for ACL reconstruction.

**Keywords:** Reinforced bone-anterior cruciate ligament-bone, allograft, anterior cruciate ligament reconstruction, biomechanics

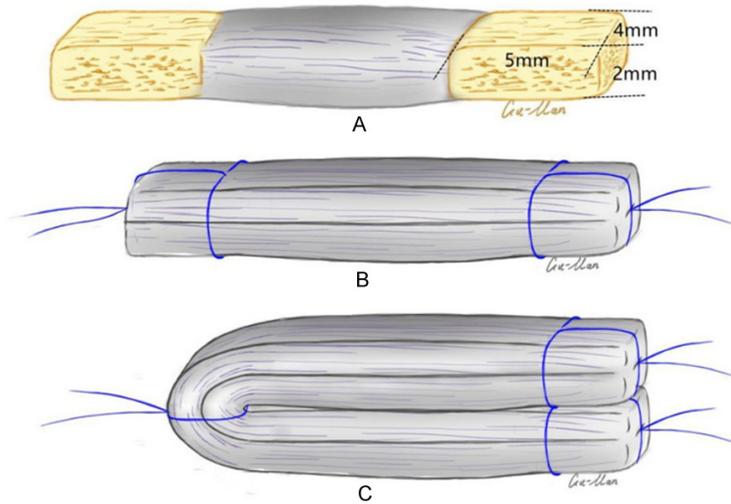
## Introduction

The incidence of knee ligament injuries, especially those of the anterior cruciate ligament (ACL), continue to increase with the advancements in sports [1]. In high school athletes, knee joint injuries account for 60% of sporting-related surgeries [2, 3] and ACL injuries account for around half of all knee injuries [4]. Over 120,000 ACL injuries occur every year in the United States, mostly in athletes in high school and college, presumably because of their increasing participation in sports-related events [5]. Therefore, ACL reconstruction becomes a more frequently performed operation in the young active patient [6]. However, it is well known that ACL surgery is high-risk procedure with complications [7-9]: there was a 13%

overall incidence of graft failure, which was higher in chronic (15%) than in acute injuries (9%); there was a 17% incidence of flexion loss greater than 10° and a 6% incidence of extension loss greater than 5°; quadriceps weakness, assessed with the one-leg hop test, was present in 27%. Now there is an increasing desire on the part of surgeons and patients alike for not only to activities of daily living but also a more rapid return to sporting activities. Hence, improvements in ACL reconstructive surgery and better recovery of knee joint function have remained important research topics in contemporary science.

The autogenous bone-patellar tendon-bone (BPTB) graft is used as a gold standard graft for ACL reconstruction [10]. The result of ACL

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**Figure 1.** A: The B-ACL-B allograft, B: The 2-stranded ST autograft, C: The 4-stranded ST autograft.

reconstruction with a BPTB autograft is excellent in midterm and long-term studies but an important concern is the fact that the prevalence of donor-site morbidity especially the anterior knee pain (AKP) [6, 8, 9]. Artit et al. [10], Arendt et al. [11] and O'Brien et al. [12] reviewing BPTB reconstructions, reported a 86%, 55% and 37% incidence of AKP, respectively. And in the long-term study of BPTB reconstructions by Johnson et al. [13] 13% of the knees failed because of patellofemoral problems. In addition, the ultimate strength of BPTB graft is weaker, and this will significantly increase the risk of failure in ACL reconstruction. A typical 10-mm BPTB graft for ACL reconstruction is 2,977 N [14] and an intact ACL is 2,160 N [15]. However, it is known that the BPTB graft have to go through the postoperative "ligamentization" process, during which initial graft strength is significantly lost and the ultimate strength becomes inferior to the native ACL [16]. And related researches report that it causes graft rupture in about 7% to 13% of ACL reconstructions [17], and laxity failure in 6% to 9% [18].

Recently, many randomized clinical trials demonstrated that ACL reconstruction with 4-strand hamstring tendons (4-SHT) provides patients with an excellent midterm and long-term clinical outcome after surgery compared to BPTB graft [19-21]. This is mainly because that an evenly tensioned 4-SHT graft has been report-

ed to be about 4,090 N [22], far more stronger than an intact ACL [15]. However, this technique has its limitations: from a biologic standpoint, it is well accepted that healing of the tendon to bone is more difficult to achieve and requires more time (usually eight to twelve weeks) than does healing of bone to bone (usually four to six weeks), which is more likely to cause the graft rupture and laxity in short-term clinical outcome [23, 24].

And most importantly, neither the 4-SHT graft nor the BPTB graft can replicate the complex anatomical morphology of the native ACL or the B-ACL-B

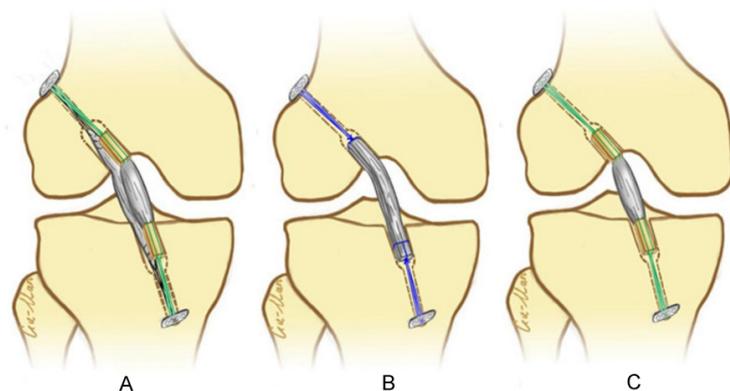
allograft. Although the B-ACL-B allograft has the advantages of anatomical morphology and bone-to-bone healing, the graft cannot withstand the forces of normal function of the knee joint after "ligamentization" process [16, 25-28]. It is for this reason that simple B-ACL-B allografts are not used in the clinic. Indeed, in their animal experiments, Jackson et al. [28] found that the B-ACL-B allograft had greater laxity and resulted in 70% lower tensile strength in the reconstructed knees as compared with the controls at 1 year after implantation. Therefore, to improve the utility of the B-ACL-B allograft for ACL reconstruction, we presented a new technique that reinforces the B-ACL-B allograft with a 2-stranded semitendinosus tendon (ST) autograft (RB-ACL-B), combining the theoretical anatomical advantage of the B-ACL-B allograft with the strength of the 2-stranded ST autograft. The aim of the present study was to compare the biomechanical properties of the RB-ACL-B allograft with that of the 4-stranded ST and B-ACL-B grafts to ascertain its potential clinical utility in ACL reconstruction. The native ACL of the contralateral limb was used as the normal control.

### Materials and method

#### Animals

Seventy-five skeletally mature, male and female, New Zealand white rabbits were obtained from a licensed laboratory animal

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**Figure 2.** A: The operation schematic diagram of RB-ACL-B group, B: The operation schematic diagram of ST group, C: The operation schematic diagram of B-ACL-B group.

dealer. Our study was approved by the institutional review board. The body weight varied from 2.70 to 3.50 kg (mean  $\pm$  standard deviation,  $3.14 \pm 0.37$  kg). Thirty rabbits were used for B-ACL-B allograft preparation, and the remaining 45 rabbits were divided into three groups for reconstructive surgery (B-ACL-B, RB-ACL-B and ST;  $n = 15$  rabbits). At 16, 32, and 48 weeks after surgery, five rabbits from each group were sacrificed for biomechanical testing.

### Graft preparation

**Allograft preparation:** The body weights of the donor rabbits were recorded before allograft preparation. Under anesthesia, we stripped the hind limbs of all musculature, joint capsule, collateral ligaments, menisci, and posterior cruciate ligament to retain the ACL. We then used an osteotome to cut bone blocks that retained the tibial and femoral insertions of the ACL as well as the anterior-medial and posterior-lateral bundles. The bone blocks were then trimmed into rectangular blocks, measuring approximately 5 mm (distal to proximal)  $\times$  4 mm (medial to lateral)  $\times$  2 mm (anterior to posterior) (**Figure 1A**). Both ends of the bone blocks were marked to ensure correctly orientation of the B-ACL-B allograft in the bone tunnels during replacement.

During harvesting of the bone blocks, however, we frequently noted damage to the lateral part of posterior-lateral bunch of the ACL that incorporated the femoral insertion. For this reason,

we positioned the 2-stranded ST autograft at this posterior-lateral defect of the ACL allograft to replace this missing portion of the tissue. We hypothesized that this could reduce the risk of rupture to the B-ACL-B allograft and improve the overall strength of the RB-ACL-B allograft. The allograft was washed with pulsating 0.9% sterile NaCl. Allografts were blotted dry and placed in screw-capped plastic tubes and labeled as left or right. Allografts were stored at  $-70^{\circ}\text{C}$  for at least 2 weeks before implantation.

**Autograft preparation:** Autologous tissues were collected at the time of implantation to prepare the 2-stranded and 4-stranded ST autografts. The thickness and width of the rabbit ACL is about 2 mm and 4 mm, respectively. Therefore, we prepared the autographs to a similar thickness, width, and length as the original ACL and the prepared the B-ACL-B allograft. Two bundles of autografts were removed from the ST of each rabbit, and revised to samples of 1 mm thickness and 4 mm width with surgical scissors and a knife blade, respectively. The length was matched to the length of the B-ACL-B allograft being used in that rabbit. The two autografts were then stitched together with non-absorbable sutures (Ethicon; **Figure 1B**).

Preparations for the 2-stranded and 4-stranded ST autografts were almost identical, except that the length of the 4-stranded ST was initially twice as long as the 2-stranded ST, before it was superimposed, folded, and stitched together with non-absorbable sutures to create a 4-stranded ST of the same length as the 2-stranded ST (**Figure 1C**).

### Surgical technique

**RB-ACL-B group:** The rabbit's ACL is too small to be measured accurately with calipers, so we matched the weights of donors and recipients for allograft selection. The body weights of the recipients were recorded before ACL reconstruction. The rabbits were anesthetized and maintained with pentobarbital sodium and premedicated with an intramuscular dose of xylocaine. Using aseptic technique, an incision was

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**Table 1.** Biomechanical comparison of the four groups at the 16<sup>th</sup> week after postoperation

Group	Maximum Force (N)	Maximum Energy (N.M)	Stiffness (N/mm)	Maximum Elongation (mm)
RB-ACL-B	120.82 ± 18.61*	150.15 ± 19.61*	44.89 ± 5.34*	3.08 ± 0.12
ST	94.31 ± 12.51*	119.90 ± 18.48*	34.80 ± 3.69*	3.03 ± 0.17
B-ACL-B	66.05 ± 15.04*	89.74 ± 15.52*	24.75 ± 5.17*	2.66 ± 0.06*
NC	292.93 ± 12.58	321.41 ± 20.32	100.68 ± 1.57	3.13 ± 0.12

\*Significant difference compared with the control group (NC group) ( $P < 0.05$ ).

made in the center of the knee joint, the patella was luxated to the medial side, and the infrapatellar fat pad was retracted to expose the ACL. The femoral and tibial insertions of the ACL were carefully transected, removing all possible ligamentous tissue. Using a 2 mm drill bit, the femoral and tibial tunnels were drilled to exit at the center of the anatomic insertion points of the ACL. A 5 mm drill bit was then used (from the inside to the outside of the joint) to expand the tunnels to a sufficient diameter and depth of 5 mm to accommodate the RB-ACL-B allograft. The joint was then lavaged with sterile 0.9% NaCl for 30 sec.

The B-ACL-B allograft was prepared for insertion by placing two high-strength, non-absorbable sutures of Ethicon through each of the bone plugs of the allograft. The sutures were then passed into the femoral and tibial tunnels, respectively, and the B-ACL-B allograft was pulled into place. Care was taken to replicate the normal spatial orientation of the ACL by visualizing the fiber orientation. The autograft was similarly prepared and inserted into place. The 2-stranded ST autografts were positioned at the posterior-lateral portion of the B-ACL-B allograft, as described above. Sutures of the allograft and autograft were tensioned usually with the knee in about 60° flexion and tied over buttons at the extraarticular exits of the tunnels (**Figure 2A**). The RB-ACL-B allograft filled the bone tunnels and produced the appropriate amount of extrusion. Sufficient tension was applied to the RB-ACL-B allograft to eliminate any anterior displacement of the tibia. The synovium, joint capsule, and skin were reapproximated with surgical sutures.

*ST group:* All steps for the ST group were the same as that described for the RB-ACL-B group, except that a 4-stranded ST autograft was used in place of the RB-ACL-B graft (**Figure 2B**).

*B-ACL-B group:* All steps for the B-ACL-B group were the same as that described for the RB-ACL-B group, except that a 2 mm drill bit rather than a 5 mm drill bit was used to expand the tunnels to a depth of 5 mm to accommodate the B-ACL-B allograft (**Figure 2C**).

*Biomechanical testing:* For a confidence level of 95% ( $\alpha = 0.05$ ) and to achieve a power (1- $\beta$ ) of 80%, we required a sample size of 15 rabbits per group. At weeks 16, 32, and 48 post-operatively, 5 rabbits from each group were euthanized, and the knee joint, including the distal femoral and proximal tibial regions, was extracted using a wire saw. The surrounding soft tissue was removed and the graft retained. The ACL from the contralateral limb (NC group) was harvested at the same time. Specimens were double-bagged and stored at -70°C for at least 2 weeks.

For biomechanical testing, we evaluated differences in maximum force (N), maximum energy (N.M), stiffness (N/mm) and maximum elongation (mm). The tibia and femur of each specimen were mounted in grips, and the graft fiber orientation visually aligned vertically under the loading axis. Tensile failure tests were conducted in a materials-testing machine at room temperature by elongating the specimen at a strain rate of 1000 mm per min until failure. A linear variable displacement transducer on the actuator monitored the actuator travel, and a strain gauge metal clip attached directly to the tibia and femur provided the relative displacement of the bone ends. A laboratory mini-computer was used to initiate the test and store the force, actuator motion, and clip gauge data. Structural parameters (ligament stiffness in the linear loading region, maximum load prior to failure, and energy and elongation to maximum load) were calculated from load-displacement curves.

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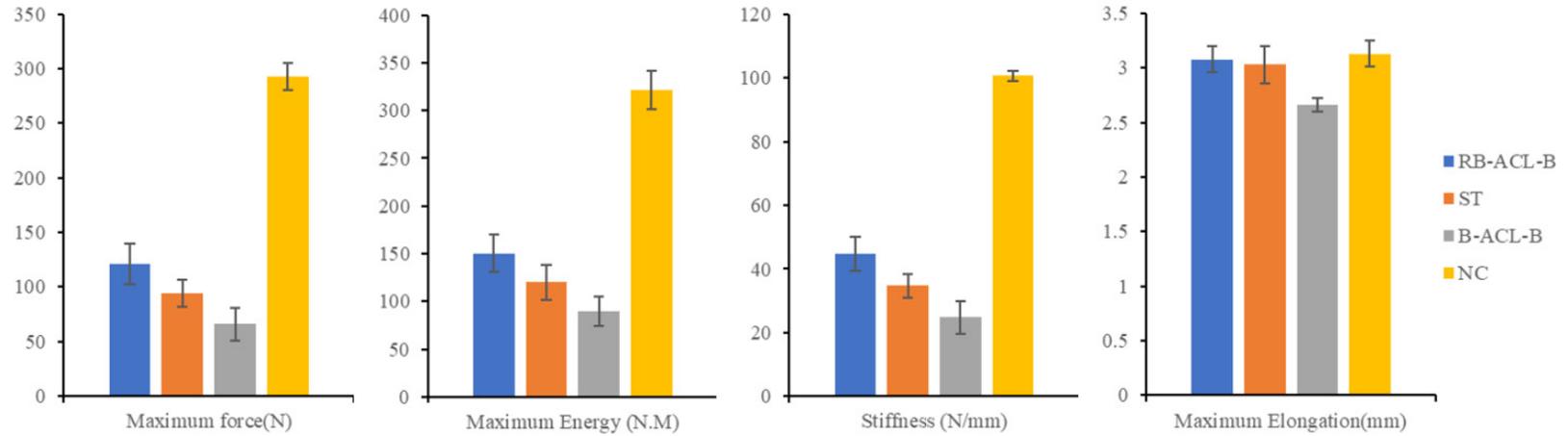


Figure 3. Biomechanical comparison of the four groups at the 16<sup>th</sup> week after postoperation.

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**Table 2.** Biomechanical comparison of the four groups at the 32<sup>nd</sup> week after postoperation

Group	Maximum Force (N)	Maximum Energy (N.M)	Stiffness (N/mm)	Maximum Elongation (mm)
RB-ACL-B	180.88 ± 18.44*	204.89 ± 20.00*	62.31 ± 3.98*	2.97 ± 0.16
ST	141.08 ± 15.29*	163.17 ± 16.55*	48.55 ± 3.59*	3.01 ± 0.13
B-ACL-B	104.39 ± 19.68*	124.62 ± 20.03*	36.84 ± 5.24*	2.77 ± 0.15*
NC	310.84 ± 20.06	331.16 ± 22.49	105.01 ± 4.01	3.07 ± 0.16

\*Significant difference compared with the control group (NC group) (P < 0.05).

## Statistical analysis

Statistical analyses were performed using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA). A Kolmogorov-Smirnov goodness-of-fit test was used to test the normality of the data. Levene's test was used to assess for homogeneity of variance. Evaluations of the maximum force, maximum energy, stiffness and maximum elongation were made with two-way analysis of variance (ANOVA), with surgical treatment and time as factors. Dunnett t-test was used to compare values between three different surgical groups and the control group. Data are shown as the mean ± standard deviation (SD). Statistical significance was set at P < 0.05.

## Results

We tested the biomechanical properties of the grafts at three time points after surgery (week 16, 32 and 48) and compared these values with the native ACL of the contralateral limb as a control. The data are presented as a percentage of that of the native control (NC, 100%).

### Sixteen weeks after surgery

The biomechanical properties of the four groups at week 16 after surgery are shown in **Table 1** and **Figure 3**. We found the maximum force withstood by the grafts was 41%, 31%, and 23% of the NC group for the RB-ACL-B, ST, and B-ACL-B groups, respectively. The maximum energy was 47%, 36%, and 28%, and the stiffness was 45%, 33%, and 25%, respectively, of the NC group. Overall, we found significant differences between the experimental groups and the NC group (P < 0.05) and also significant differences between the RB-ACL-B group and the two other experimental groups (P < 0.05). Maximum elongation was 98%, 97%, and 85% of the NC group, respectively, with a significant difference detected only between the NC group and B-ACL-B group (P < 0.05).

### Thirty-two weeks after surgery

At week 32 (**Table 2; Figure 4**), the maximum force for the RB-ACL-B, ST, and B-ACL-B groups increased from that at week 16 to 58%, 45%, and 34% of the NC group, respectively. Similarly, we measured increases in the maximum energy (62%, 49% and 38% of the NC group, respectively) and stiffness (59%, 46%, and 35% of the NC group, respectively), with significant differences measured between the NC and the experimental groups, as well as between the RB-ACL-B and the other two experimental groups. The maximum elongation was 97%, 98%, and 90% of the NC group, with a significant difference again measured only between the NC group and B-ACL-B group (P < 0.05).

### Forty-eight weeks after surgery

By the 48th week after surgery, the maximum force was 68%, 55%, and 38%; the maximum energy was 70%, 59%, and 42%; and the stiffness was 68%, 55% and 42% of the NC group for RB-ACL-B, ST, and B-ACL-B groups, respectively (**Table 3; Figure 5**; P < 0.05). Significant differences were noted for the NC group as compared with the experimental groups and for the RB-ACL-B group over the ST and B-ACL-B groups (P < 0.05). Maximum elongation was 101%, 96%, and 86% of the NC group, with a significant difference detected between the NC group and B-ACL-B group (P < 0.05).

## Discussion

The structure of the ACL is complicated, divisible by its fiber lines into anterior-medial and posterior-lateral groups [29, 30]. Morey [31] and Kondo [32] in their respective works have both shown that a double-bundle anatomic ACL reconstruction better resembles the fiber direction and complex structure of a normal ACL as compared with a single-bundle graft, and cite significant improvements in the postoperative functional outcomes, kinematic restoration,

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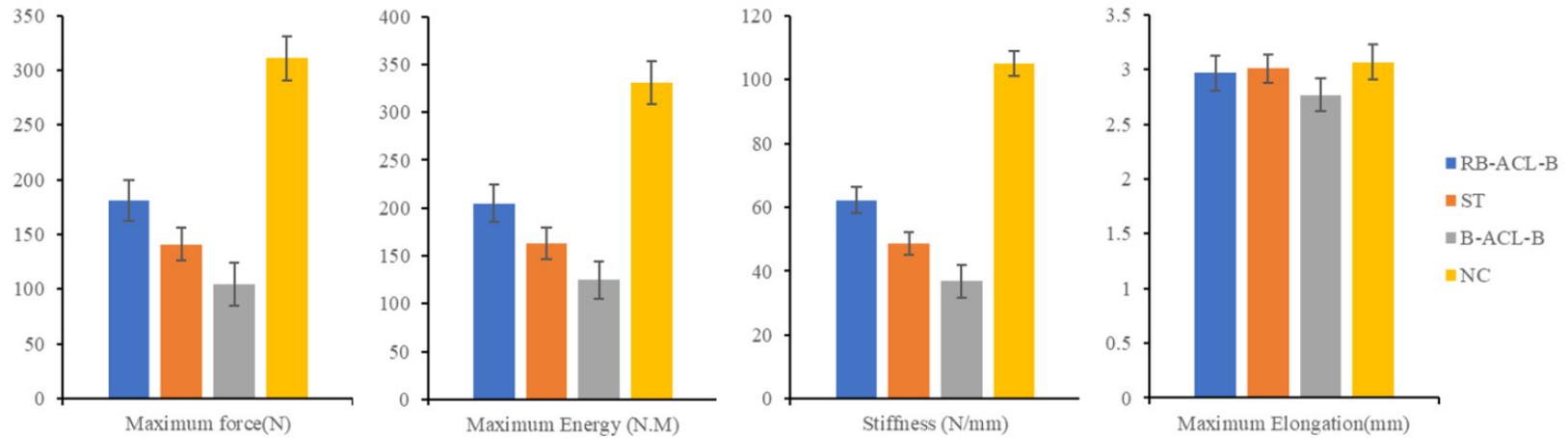


Figure 4. Biomechanical comparison of the four groups at the 32<sup>nd</sup> week after postoperation.

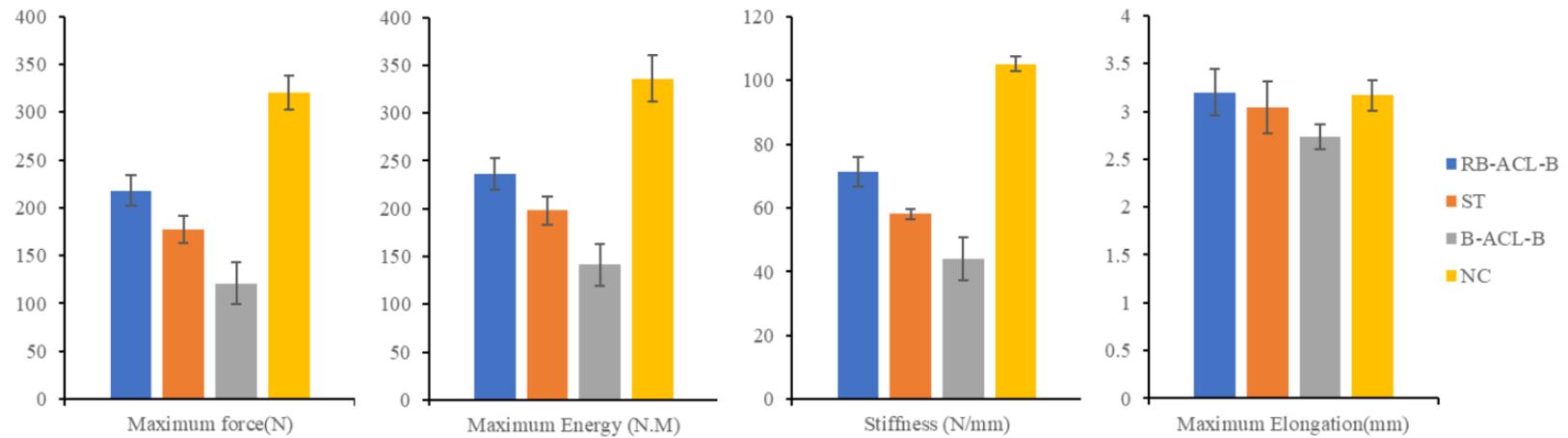


Figure 5. Biomechanical comparison of the four groups at the 48<sup>th</sup> week after postoperation.

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**Table 3.** Biomechanical comparison of the four groups at the 48<sup>th</sup> week after postoperation

Group	Maximum Force (N)	Maximum Energy (N.M)	Stiffness (N/mm)	Maximum Elongation (mm)
RB-ACL-B	217.93 ± 15.81*	236.34 ± 16.63*	71.23 ± 4.64*	3.20 ± 0.24
ST	177.35 ± 14.23*	198.19 ± 14.88*	58.12 ± 1.62*	3.04 ± 0.27
B-ACL-B	120.76 ± 21.84*	141.44 ± 21.87*	44.05 ± 6.81*	2.73 ± 0.13*
NC	320.16 ± 17.56	335.99 ± 24.43	105.14 ± 2.24	3.17 ± 0.16

\*Significant difference compared with the control group (NC group) ( $P < 0.05$ ).

and clinical stability of the graft. In our study, the 4-stranded ST autograft woven together would be unable to imitate the normal anatomical morphology of the ACL, and this would lead to an overall reduction in the efficacy of the ACL reconstruction. However, the RB-ACL-B allograft not only maintains the normal anatomical morphology of the ACL, but also provides a physiological scaffold for collagen regeneration, more closely restoring the histological and mechanical properties of the joint [25, 26, 33].

The RB-ACL-B allograft incorporates both bone-to-bone and tendon-to-bone healing, but the priority is given to the bone-to-bone healing, as it is sturdier postoperatively and heals quicker than a tendon-to-bone repair [23, 24]. This enhances the overall stiffness of the implant and enables earlier rehabilitation to restore knee joint function without postoperative complications [34, 35]. Furthermore, histological assessment of the B-ACL-B allografts shows the presence of a regularly oriented, dense connective tissue and complete revascularization of the ligament and bone plugs, resembling a normal ligament [25, 28].

The RB-ACL-B allograft and the 4-stranded ST autograft would theoretically have a similar strength intensities; however, our experimental results showed that the biomechanical properties of the RB-ACL-B allograft were better than those of the ST graft. We show that at weeks 16, 32, and 48, the maximum force for the ST group was 74%, 78%, and 81% of that of the RB-ACL-B group ( $P < 0.05$ ). Similarly, the energy to maximum force and the stiffness values for the ST group ranged from around 70% to 80% of those values for the RB-ACL-B group values over the three time points (energy to maximum force 77%, 80%, and 84%; stiffness 73%, 78%, and 82%;  $P < 0.05$ ). We attribute these advantages of the RB-ACL-B allograft to its complete anatomical morphology of a normal ACL and the main healing way of bone-to-bone.

Noyes and others used the medial one-third and middle one-third of the BPTB for ACL reconstructive surgery, and found that the maximal tensile load of the middle one-third was higher than that of the medial one-third [36]. Others have shown that the larger cross-sectional area of transplanted ligament will provide the greater tension of the reconstructed ligament and the better stability of the knee joint postoperatively [37-39]. The overall cross-sectional area of RB-ACL-B allograft was increased with the 2-stranded ST and it resulted in a greater tension and maximum tensile load: maximum force of the B-ACL-B group was 55%, 58%, and 55% of that of the RB-ACL-B group, and the stiffness was 55%, 59% and 62% at 16, 32 and 48 weeks (both,  $P < 0.05$ ). Therefore, we surmise that the RB-ACL-B allograft offers important anatomical morphology of a native ACL, bone-to-bone healing and satisfactory initial strength of graft to improve its biomechanics properties for ACL reconstruction over the use of the other graft types.

The knee joint in the rabbit is small; therefore, we stabilized the graft using high-strength non-absorbable sutures (Ethicon) tied over buttons rather than with an interference screw. However, others have shown that the gold standard for fixation is via extrusion screw theory, which offers strong hole extrusion [40-42]. Stable fixation of a graft will significantly improve its biomechanical properties and reduce the risk of ACL reconstruction failure [43, 44]. And compared with unprotected grafts, the grafts protected with a ligament-augmentation device will show significant improvements in strength [27, 45]. Therefore, we could reasonably speculate that, if the knee joint of the rabbit was large enough to use extrusion screws for fixation, the biomechanical properties would be better than those fixed with non-absorbable sutures. This likely explains why the biomechanical properties of

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the RB-ACL-B allograft were significantly lower than those of the NC group in our study.

Another limitation is that the rabbit ACL is too small to be measured accurately with calipers. For this reason, we sought to match donor and recipient rabbits based on similar weights. However, in some cases, this led to length mismatch between the donor and recipient. A graft that is longer than the recipient's ACL length can cause instability and accentuate stretching of the ligament, whereas a shorter allograft would be placed under considerable stress and would either stretch to accommodate the length requirement or rupture [25]. In animal studies, we can prepare a large selection of donor grafts to ensure a proper fit; however, this is not a solution for clinical cases. In the clinic, because the recipient's ACL cannot be measured directly, it is important to use anatomical landmarks or the measure of the length of the contralateral ligament using magnetic resonance imaging to ensure proper size matching.

In conclusion, good biomechanical properties are achievable using RB-ACL-B allografts prepared using B-ACL-B allografts and ST auto-grafts in rabbit ACL reconstruction. These combined grafts offer superior strength over B-ACL-B or ST grafts alone. In the clinic, a reinforced B-ACL-B allograft may be an ideal substitute for ACL reconstruction.

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### Disclosure of conflict of interest

None.

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