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Original article

Comparison of postoperative biomechanical function between anatomic double-bundle and single-bundle ACL reconstructions using calcium phosphate-hybridized tendon grafts in goats

H. Mutsuzaki[a,*], H. Fujie[b], H. Nakajima[c], M. Fukagawa[b], S. Nomura[c], M. Sakane[d]

[a] *Department of Orthopaedic Surgery, Ibaraki Prefectural University of Health Sciences, 4669-2 Ami Ami-Machi, Inashiki-gun, Ibaraki 300-0394, Japan*
[b] *Biomechanics Laboratory, Faculty of System Design, Tokyo Metropolitan University, 6-6 Asahigaoka, Hino, Tokyo 191-0065, Japan*
[c] *Department of Agriculture, Ibaraki University, 3-21-1 Chuuou, Ami, Ibaraki 300-0393, Japan*
[d] *Department of Rehabilitation Medicine, Tsukuba Gakuen Hospital, 2573-1 Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan*

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ABSTRACT

Background: Calcium phosphate (CaP)-hybridized tendon grafts improved biomechanical function compared with untreated grafts after single-bundle (SB) anterior cruciate ligament (ACL) reconstruction. The purpose of this study was to compare the biomechanical function between anatomic double-bundle (DB) and single-bundle (SB) ACL reconstructions using CaP-hybridized tendon grafts at 6 months postoperatively in goats.
Hypothesis: We hypothesized that the postoperative biomechanical function in the DB group will be better than that in the SB group.
Materials and methods: Knee kinematics and *in situ* forces in the grafts under applied anterior tibial load (ATL) of 50 N and internal tibial torque (ITT) of 2.0 Nm at full extension, and 60° and 90° of knee flexion, and the histology of the tendon–bone interface were compared between the DB group ($n = 6$) and SB group ($n = 6$).
Results: The *in situ* forces under ATL in the DB group at full extension and 90° of knee flexion were greater than those in the SB group. The *in situ* forces under ITT in the DB group at full extension and 60° of knee flexion were greater than those in the SB group. The *in situ* forces on the posterolateral bundle of the grafts under ATL and ITT in the DB group at full knee extension were greater than those on the posterior half of the grafts in the SB group. The histology did not differ significantly between the groups.
Conclusions: Although CaP-hybridized tendon grafts were used in both groups, the *in situ* forces under ATL and ITT in the DB group were greater than those in the SB group at 6 months postoperatively. The posterolateral bundle of the grafts in the DB group acted effectively against both ATL and ITT at full extension. The tendon-to-bone healing was similar in both groups.
Study design: Controlled laboratory study. Level 2.

1. Introduction

Calcium phosphate (CaP)-hybridized tendon grafts prepared by an alternate soaking process improved tendon-to-bone healing [1]. The CaP hybridization technique is a method that involves alternate soaking of tendon grafts in a Ca solution and a P solution for 30 seconds each, with the cycle repeated 10 times [2,3]. Using this technique, direct bonding areas without fibrous layers were observed between the CaP-hybridized tendon graft and the bone at 2–3 weeks after implantation in rabbits [2,3]. In a comparison of the CaP-hybridized tendon grafts and untreated grafts after non-anatomic single-bundle (SB) ACL reconstruction in a goat model at 1 year postoperatively, better anterior stability and greater *in situ* forces were observed in the CaP group [4]. Moreover, at 6 months after anatomic SB ACL reconstruction, greater *in situ* forces under applied anterior tibial loads (ATL) were observed in the CaP group compared with the control group [5]. In the CaP group, better tendon-to-bone healing involving a larger cartilaginous layer, direct bonding area and smaller nonbonding gap area at the tendon–bone interface, was observed compared with the control group [4,5].

For further functional improvement, we proposed the combination of anatomic double-bundle (DB) ACL reconstruction and use of a CaP-hybridized tendon grafts. The human ACL is composed

* Corresponding author.
E-mail address: mutsuzaki@ipu.ac.jp (H. Mutsuzaki).

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of anteromedial (AM) and posterolateral (PL) bundles with different biomechanical functions. The AM bundle is taut during knee flexion and the PL bundle tightens in knee extension [6]. The PL bundle also plays a significant role in stabilization of the knee against a combined rotatory load [7]. In cadaveric human knees, the biomechanical function after anatomic DB ACL reconstruction more closely approximated the normal ACL compared with that after SB ACL reconstruction [8].

The purpose of this study was to compare the biomechanical function and histology of the tendon–bone interface between anatomic DB and SB ACL reconstructions using CaP-hybridized tendon grafts at 6 months postoperatively in goats. We hypothesized that the biomechanical function in the DB group will be better than that in the SB group and that the tendon-to-bone healing in the DB and SB groups will be similar at 6 months postoperatively.

2. Materials and methods

Eighteen skeletally mature female Saanen breed goats (40–60 kg) were used in the study (DB, $n = 6$; SB, $n = 6$). Data from an intact-ACL group ($n = 6$) was used as reference values for the biomechanical analysis. All animal experiments and breeding were performed under conditions approved by the ethics committees of the University of Tsukuba. All experimental activities were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the ARRIVE guidelines.

2.1. Graft preparation

The flexor digitorum longus tendon (total length: 16 cm) was harvested for use as the tendon graft in the right knee. The tendon was prepared by quadrupling the tendon length to 40 mm with a diameter of 7.0 mm for each SB reconstruction [5]. For each DB reconstruction, the tendon was cut in half to the same length to prepare an AM graft and a PL graft. The two tendons were prepared by doubling the tendon length to 40 mm with a diameter of 4.5 mm. The tibial end of the graft was secured using the Krackow technique with No. 2 nonabsorbable sutures, and the double No. 2 nonabsorbable suture tied over the endobutton was passed through the looped femoral end of the graft.

The CaP-hybridization method was the same as that described in our previous reports [2–5]. Briefly, the grafts were soaked in 100 mL of a Ca solution for 30 seconds and then soaked in 100 mL of $NaHPO_4$ solution for 30 seconds, with the cycle repeated 10 times.

2.2. Anatomical SB ACL reconstruction

The surgical technique was the same as that described in our previous report [5]. The ACL was completely resected. We then cleated 7.0-mm-diameter femoral and tibial bone tunnels anatomically and passed the tendon graft through the bone tunnels. The graft was secured on the femoral side with the endobutton, tensioned with a manually-applied maximal force and fixed on the tibial side by tying the sutures around a 4.5-mm cortical screw (Fig. 1).

2.3. Anatomical DB ACL reconstruction

We performed the DB ACL reconstruction in the same manner as the SB reconstruction until the ACL resection. We then created 4.5-mm-diameter femoral bone tunnels from the center of the femoral insertion of the AM and PL bundles of the ACL to the lateral cortex of the distal femur, as well as a 4.5-mm-diameter tibial bone tunnel from the center of the tibial insertion of the AM and PL bundles of the ACL to the medial cortex of the proximal tibia. The tendon graft was passed through the bone tunnels. Each graft was secured on the femoral side with the endobutton. The two grafts were simultaneously tensioned with a manually-applied maximal force and fixed on the tibial side by tying the sutures together around a 4.5-mm cortical screw at 45° of knee flexion (Fig. 2).

Postoperatively, the goats were allowed free activity in a 50-m^2 cage. The goats were euthanized at 26 weeks after surgery. The 18 knee specimens were harvested, sealed in double plastic bags and immediately stored at −20 °C until 24 hours prior to biomechanical analysis [4,5].

2.4. Biomechanical analysis

We performed the biomechanical analysis using the same testing methods described in our previous study [4,5]. The robotic testing system (FRS2010, Technology Services, Chino, Japan) consisted of a 6-degrees-of-freedom (DOF) manipulator, servomotor controllers and a control computer. The femoral clamp was fixed to the lower mechanism and the tibial clamp was fixed to the upper mechanism in series with a universal force-moment sensor (UFS) (SI-660-60; ATI, Apex, NC, USA). The hardware and software performances were described in our previous report [9]. The robotic system was capable of controlling the displacement and force/moment applied to the knee in 6-DOF based on a mathematical description of knee kinematics and kinetics [10,11]. Thus, the testing system permitted measurement of the triplanar knee kinematics and *in situ* forces within the ACL replacement graft while the knee was subjected to externally applied loads [12,13].

After determining the path of passive flexion–extension of the knee, the robotic/UFS testing system was used in force-control mode to determine the 5-DOF knee kinematics in response to a 50-N anterior–posterior (A-P) tibial load and a 2.0-Nm internal–external tibial torque at full extension and 60° and 90° of knee flexion [4,5]. In the intact-ACL and DB groups, the AM bundle was transected, followed by the PL bundle. In the SB group, the anterior half of the ACL graft (AH) was transected, followed by the posterior half of the ACL graft (PH). Under these conditions, the testing system reproduced the pre-transection three-dimensional path of knee motion while again measuring the 6-DOF forces/moments. The *in situ* forces in the ACL or ACL graft during flexion–extension, A-P drawer tests and internal tibial rotation tests were calculated. The differences in these forces and moments during the fifth cycle between the intact and transected knee states were determined using the principle of superposition [14].

2.5. Histological analysis

After the biomechanical analysis, the specimens were fixed in 10% neutral-buffered Formalin, decalcified, and embedded in paraffin. Hematoxylin, eosin and safranin-O staining were performed. At the joint aperture site of the femoral and tibial interfaces, the total area of red safranin-O-stained glycosaminoglycans (GAGs) in the cartilage layer and the total length of nonbonding gap area from the joint were measured in the sagittal plane [4–6]. The ligament tissue maturation index [15] was used to evaluate the maturation of the tendon grafts. In the DB group, the AM bundle and PL bundle were evaluated. In the SB group, the AH and PH were evaluated.

2.6. Statistical analyses

The biomechanical data and histological analyses were compared by Student's *t*-tests. *P* values less than 0.05 were considered statistically significant.