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What Is the Best Candidate Allograft for ACL Reconstruction? An in Vitro Mechanical and Histologic Study in a Canine Model

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Abstract

The knee joint is generally characterized by very low friction and high wear resistance. Several previous studies have compared ACL with the commonly used allografts from tensile properties perspective. No study has reported about the graft tendons from a frictional perspective, which is an important parameter for ACL functional performance. Twenty hind legs were used to harvest FDP tendon, ACL, ACH, and patellar tendon. Samples were evaluated with surface friction testing, indentation testing for tendon compressive moduli, lubricin immunohistochemistry, and histologic analysis. Frictional force of FDP tendon and ACL was significantly less than that of patellar tendon and ACH at first and fifth cycles. At the tenth cycle, the FDP tendon, ACL, and ACH showed significantly less frictional force than patellar tendon; after 100 cycles, the FDP tendon and ACL showed significantly less frictional force than patellar tendon. The compressive moduli of the FDP tendon, ACL, and ACH were significantly greater than that of patellar tendon. Histologic results showed that FDP tendon and ACL had a smooth surface with a thin layer of epitenon cells; patellar tendon and ACH had a rough surface and a layer of paratenon. Lubricin was found on the surface and extracellular matrix of FDP tendon and ACL. There was only limited lubricin expression on the surface and extracellular matrix of the ACH and patellar tendon. The FDP tendon has friction force and lubricin expression similar to those of native ACL. However, patellar tendon and ACH show higher friction force and less lubricin expression than ACL.

Keywords

Tendon; Ligament; Friction testing; Lubricin; ACL reconstruction

Introduction

Although ligaments and tendons share many characteristics, they are fundamentally different in order to meet different functional needs. Tendons attach muscle to bone and produce motion across the joints; ligaments connect bone to bone and control motion of the joints. On the basis of the surrounding environment, tendons can be differentiated into intrasynovial tendons, such as flexor digitorum profundus (FDP) and extrasynovial tendons, such as

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Conflict of Interest

None of the authors of this manuscript have any conflicts of interest to disclose

Achilles tendon (ACH) and patellar tendon (PAT); ligaments can be also differentiated into intra-articular ligament, such as anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) and extra-articular ligament, such as medial collateral ligament (MCL) (Carpenter and Hankenson, 2004).

ACL reconstruction has become the gold standard for treating ACL rupture to prevent knee instability, with an estimated 100,000 ACL reconstructions performed annually in the United States (Griffin et al., 2000). With improved donor screening and with modern procurement and sterilization techniques, use of allograft for ACL reconstruction has increased markedly (Vangsness et al., 2003). The commonly used allograft includes anterior tibial tendon, posterior tibial tendon, ACH, quadriceps tendon, bone–patellar tendon–bone, peroneus longus tendon, and hamstring tendon (Fu et al., 2009). A recent study by Kropf et al. (Kropf et al., 2013) found that ACL-PCL contact occurred in 25% of native knees. After double-bundle ACL reconstruction, contact between the ACL graft and the PCL occurred in 75% of reconstructed knees. Furthermore, Iriuchishima et al. (Iriuchishima et al., 2012) found that the ACL-PCL impingement pressure was up to 0.33 MPa in normal porcine knee. These findings are suggestive that friction or abrasion might exist between ACL and PCL in some native knees and ACL reconstructed knees. Several previous studies have compared ACL with the commonly used allografts including hamstring tendon, bone–patellar tendon–bone, and ACH from tensile properties perspective (Cooper et al., 1993; Hamner et al., 1999; Noyes et al., 1984; Woo et al., 1991; Wren et al., 2001). To the best of our knowledge, no study has reported about the difference of graft tendons from a frictional perspective, which is an important parameter for ACL functional performance. The main objective of the present study was to compare the differences among ACL, FDP, ACH, and PAT with friction testing, indentation testing, and histologic techniques.

Methods

Sample preparation involved 20 hind legs harvested from 10 adult, male, mixed-breed dogs (weight, 21–26 kg) that were euthanized for other studies approved by the Institutional Animal Care and Use Committee of our institute. The third FDP tendon (N=20), ACL (N=20), middle bundle of ACH (N=20), and central one-third of PAT (N=20) were harvested immediately after death. In each group, 8 specimens were used for friction testing, 8 for indentation testing, and 4 for histologic analysis.

Friction Testing

The friction test device was designed on the basis of previously established methods (Uchiyama et al., 1997). A Simax glass rod (Friedrich and Dimmock, Inc.) with a 4-mm diameter was connected in line with an Arcus motor (Arcus Technology, Inc.). The sites of friction test are illustrated in Figure 1. The tendons and ligaments were sutured with braided suture (Cortland Line Co., Inc.) at both ends. The right of the braided suture was connected to a load cell (MDB-10; Transducer Techniques, LLC); the left side was connected to a 200-g (1.96-N) weight through a pulley (Figure 2A and B). The angle between the horizontal plane and the braided wire was set to 25° on both sides.

All samples were immersed in normal saline to avoid drying. The glass rod was rotated clockwise with 2 mm/s for 3 turns and then counterclockwise with 2 mm/s for 3 turns, which was defined as a *cycle*. Based on preliminary experiments, when the total number of cycles reaches 100 cycles, the frictional force began to stabilize. Therefore, 100 cycles was used for the present study. The frictional force was calculated from previous studies (Uchiyama et al., 1997; Uchiyama et al., 1995). Data for each cycle were separated into a forward phase and a reverse phase (Figure 2C).

The difference in force reading for each phase was calculated throughout the cycle, and *frictional force* (f) was defined as one-half of the average force over the plateau of this curve based on the following calculations. If there is no friction (f) between tendon and rolling rod, then the force measured by the load cell is equal to the weight (W). If there is friction, the frictional force is determined with the rolling direction. If the rod rolls clockwise, the force measured ($F1$) is equal to $W+f1$; if the rod rolls counter clockwise, the force measured ($F2$) is equal to $W-f2$. Therefore, $f1=F1-W$ and $f2=W-F2$. The average frictional force is $\frac{f1+f2}{2} = \frac{(F1-W)+(W-F2)}{2}$, then, $= \frac{F1-F2}{2}$. A custom MATLAB program (The MathWorks, Inc.) was developed to automatically calculate the frictional force for each cycle (Figure 2C).

Indentation Test

The indentation test was performed in accordance with the previously established method (Qu et al., 2013). Samples were cut into segments (the same site as for the friction test) to a length of 2 cm. The samples were placed in an aluminum platform with sandpaper to avoid tendon sliding. The ElectroForce 3200 test system (Bose Corp.) was used for indentation test. A 3-mm flat, nonporous indenter was brought into contact with the sample. After a preload of 0.1 N, testing was performed at a loading rate of 10 N/min to a maximum force of 5.0 N. The height of all samples measured under 0.03 N. The recorded data were used to calculate the stress-strain curve. The slope of linear region of the stress-strain curve was considered to be the compressive modulus.

Histologic Analysis

The same sites as for the friction test were used for histologic analysis. The samples were frozen in Tissue-Tek compound (Sakura Finetek) and then sectioned transversely or longitudinally at 10 μ m using a cryostat (CM1850; Leica). Sections were collected in the charged glass slides (Fisher Scientific). These slides were used for hematoxylin-eosin staining. Lubricin immunohistochemical staining was performed in accordance with previous methods (Sun et al., 2008; Sun et al., 2013). Briefly, these sections were rinsed 3 times with phosphate buffered saline (PBS) and fixed with a 4% paraformaldehyde buffer for 5 minutes. The slides were rinsed with PBS consisting of 3% Triton X-100 (Sigma Chemical) and then blocked with PBS containing 3% Triton X-100, 0.25% bovine serum albumin, and 4% normal goat serum for 30 minutes. The slides were rinsed with Tris buffer containing 0.1% bovine serum albumin and 0.5% Triton X-100 (short as TBS here). They were incubated with 1 μ g/mL anti-lubricin monoclonal antibody S6.7925 (a kind gift of Thomas M. Schmid, PhD, Rush University, Chicago, Illinois) for 2 hours at room temperature and washed with TBS. The slides were incubated with a biotinylated horse antimouse immunoglobulin G (Vectastain Elite ABC kit; Vector Laboratories, Inc.) for 30

minutes and with Vectastain ABC reagent (Vectastain Elite ABC kit) for 30 minutes. ImmPACT AEC Peroxidase Substrate (Vector Laboratories, Inc.) was applied to the slides for 10-minutes to detect lubricin. VECTOR hematoxylin (Vector Laboratories, Inc.) was used for counterstaining. Aqueous mounting medium (Vector Laboratories, Inc.) was applied to the slides. These slides were observed with an Olympus microscope (BH-2; Olympus, Tokyo, Japan).

Data Analysis

The quantitative data (frictional force and compressive modulus) were analyzed with SAS JMP software (version 10; SAS Institute Inc.) with one-way analysis of variance, followed by Tukey-Kramer post-hoc test for pairwise comparison. Statistical significance level was set at $P < .05$.

RESULTS

Frictional Force Test

The frictional force increased with the number of cycles in 4 groups (Figure 3). The mean (SD) frictional force of the FDP tendon (47.24 ± 14.40 mN) and ACL (51.98 ± 12.10 mN) was significantly less than those of PAT (336.62 ± 110.87 mN) and ACH (147.89 ± 54.73 mN) at the first cycle ($P < 0.05$). At the fifth cycle, the mean (SD) frictional force of the FDP tendon (88.73 ± 18.33 mN) and ACL (126.88 ± 50.83 mN) was also significantly less than those of PAT (414.29 ± 104.49 mN) and ACH (260.11 ± 99.11 mN) ($P < 0.05$). At the tenth cycle, the mean (SD) frictional force of the FDP tendon (132.53 ± 26.08 mN), ACL (196.69 ± 84.80 mN), and ACH (311.89 ± 124.78 mN) was significantly less than that of PAT ($442.12 [106.69]$ mN) ($P < 0.05$). At cycle 100, the mean (SD) frictional force of the FDP tendon (397.26 ± 39.98 mN) and ACL (396.53 ± 90.59 mN) was significantly less than that of PAT (571.06 ± 132.50 mN) ($P < 0.05$).

Indentation Testing

The mean (SD) compressive moduli of the FDP tendon (4.11 ± 0.34 MPa), ACL (3.54 ± 0.32 MPa), and ACH (4.71 ± 0.38 MPa) was significantly greater than that of PAT (2.85 ± 0.43 MPa) ($P < 0.05$). In addition, the compressive modulus of ACH was significantly greater than that of ACL ($P < 0.05$) (Figure 4).

Histologic Results

The FDP tendon and ACL showed a smooth surface covered by a single layer of epitenon cells; PAT and ACH showed a relatively rough surface covered by a layer of loose paratenon. The collagen in FDP and ACL seemed denser than that of PAT and ACH (Figure 5A–D). Lubricin was highly expressed on the surface and extracellular matrix of the FDP tendon and ACL. The paratenon and extracellular matrix of ACH expressed some lubricin. However, there was only limited lubricin expression on the surface and extracellular matrix of PAT (Figure 5E–H).

Discussion

Although several studies have compared the tensile properties of the FDP tendon, ACL, ACH, and PAT (Cooper et al., 1993; Hamner et al., 1999; Noyes et al., 1984; Pring et al., 1985; Woo et al., 1991; Wren et al., 2001), this is the first study to compare their differences in frictional property, which is an important factor for ACL reconstruction. We found intrasynovial FDP tendon and intra-articular ligaments (ACL) had similar frictional properties, which was superior to extrasynovial tendon (ACH and PAT). Lubricin was expressed mainly on the surface of the FDP tendon and ACL, with less lubricin expression on the surface of ACH and PAT. The frictional force of tendon and ligament was coincided with the expression of lubricin (Sun et al., 2008; Sun et al., 2013).

Both tendons and ligaments belong to dense connective tissue histologically. The main component of tendons and ligaments is type I collagen fibers, and fibroblasts are arranged in rows between the collagen fibers. To meet different functions, tendons and ligaments gradually adapt to the surrounding environment and adjust to different mechanical properties and structural characteristics (Carpenter and Hankenson, 2004). The FDP tendon, as the intrasynovial tendon, showed less frictional force in cycles 1, 5, 10, and 100. The main function of the FDP tendon is to flex the finger or toe. Finger or toe flexion motion is frequent, and the tendons glide within a narrowing osseous-fiber pulley system. Therefore, flexion of finger or toe need to be completed with low friction (Uchiyama et al., 1997). Interestingly, in our results, the ACL showed similar frictional force as the FDP tendon and significantly less than the ACH and PAT. Histologically, the FDP tendon and ACL are covered with synovial membrane and surrounded by synovial fluid. In contrast, the ACH and PAT are extrasynovial and do not have a synovial membrane but instead has a paratenon of loose connective tissue, which possesses an inferior gliding ability and durability against abrasion.

ACL impingement either against intercondylar roof (Udagawa et al., 2014) or the posterior cruciate ligament (Iriuchishima et al., 2012) can be the cause of the clinical problems of an effusion, extension deficit, recurrent instability, or anterior knee pain (Watanabe and Howell, 1995). Kropf et al found that after double-bundle ACL reconstruction, contact between the ACL graft and the PCL occurred in 75% of reconstructed knees (Kropf et al., 2013). These data suggested that abrasion may exist between ACL and intercondylar roof or PCL during impingement while the knee is moving that may lead to fractured bundles, guillotined remnants at the tibial insertion, parallel fragmentation of an uninterrupted graft, fibrous nodule, or an extrusion of graft material at the outlet of the notch, all of which were observed by Watanabe et al upon arthroscopic follow-up (Watanabe and Howell, 1995). Although a notchplasty is often performed to avoid graft impingement, greater blood loss and possible alteration to knee kinematics caused by this procedure are concerns (Keklikci et al., 2013; Pape et al., 2001). Therefore, the frictional of the ACL graft material is an important parameter that may affect the clinical outcomes after ACL reconstruction. However, the ACL frictional properties and related surface morphologic characteristics have not been studied. The present study showed that the ACL, as an intra-articular ligament, has similar surface frictional properties, structure, and lubricin expression as the FDP tendon, an intrasynovial tendon. Further analysis showed that the frictional force of ACH was

significantly less than that of PAT. The main function of ACH is to transfer the gastrocnemius muscle contraction force to calcaneus and thereby it does not need too much gliding. The PAT connects the patella and tibia, with a role in transferring contraction force of the quadriceps to the tibia. In this study, we measured the friction of the posterior side of the central PAT where it is covered with infrapatella fat pad. No gliding exists between PAT and infrapatella fat pad, and this should account for the high frictional force.

Previous studies have directly compared the friction difference between intrasynovial tendons and extrasynovial tendons. Uchiyama et al. (Uchiyama et al., 1997) found that frictional force of the FDP tendon was significantly less than the palmaris longus tendon. Their result was consistent with our findings. However, in their study, the A2 pulley (i.e., physiological conditions) was used against tendon gliding for friction testing. Under physiological conditions, the frictional force of the FDP tendon did not increase with the increase of cycle (Ozasa et al., 2013). In the present study, a glass rod, which has a higher frictional coefficient than the tendon tissue (Sun et al., 2008; Sun et al., 2013), was used against tendon gliding, which caused the frictional force to increase with the increase of the cycle.

In addition to subject tensile load, the FDP tendons also bear the compressive force during fist or grasp activities. Iriuchishima et al. (Iriuchishima et al., 2012) found that the mean (SD) ACL-PCL impingement pressure was 0.33 (0.22) MPa in normal porcine knee. Therefore, our study used indentation testing to study the difference in compressive modulus among FDP tendon, ACL, ACH, and PAT. We found that the compressive modulus of the FDP tendon and ACL are similar.

Lubricin is distributed widely in the body, particularly in the musculoskeletal system. It has been reported to be able to reduce friction in joints and tendons. Sun et al. (Sun et al., 2008; Sun et al., 2013) demonstrated that removal of lubricin using trypsin dramatically increased the frictional force of the FDP tendon. Taguchi et al. (Taguchi et al., 2009) found that lubricin compound could reduce the gliding resistance of peroneus longus significantly. Further in vivo studies found that lubricin compound could reduce substantially the adhesions after peroneus longus and lyophilized FDP tendon allograft transplantation in a canine model (Zhao et al., 2013; Zhao et al., 2010). In the current study, lubricin was expressed mainly on the surface of the FDP tendon and ACL. Lubricin was expressed limitedly on the surface of ACH and PAT. Lubricin expression coincided with the frictional force of the tendons and ligament.

The native ACL had an ultimate load to failure of approximately 1,725 N (Noyes et al., 1984). The third flexor digitorum profundus (FDP) tendon had an ultimate load to failure of 1182 ± 213 N and a length of 183–255 mm (Pring et al., 1985). Doubled FDP tendon should own similar ultimate failure load to the native ACL. The current study showed that the FDP tendon had the closest frictional force, compressive moduli, and lubricin expression to the ACL because both are located in an intrasynovial environment. This similarity suggests that the FDP tendon may be a candidate graft for ACL reconstruction.

The current study has several limitations. First, this study used a canine model, not a human model. Many differences exist between canine and human in the histologic and anatomical structures. For example, the ACH of dogs is composed of 3 bundles; the human ACH contains only 1 bundle. Second, this study compared only the differences among the FDP tendon, ACL, ACH, and PAT from biomechanical and histologic perspectives. The microstructure difference is not clear. Further studies with scanning electron microscopy and transmission electron microscopy may help to understand the microstructure. Third, the hamstring tendon, which is a common graft for ACL reconstruction, was not included in the study because the canine model does not have typical hamstring tendons for testing. Fourth, as this was an in vitro study, we did not know whether the frictional properties of allografts would remain the same over time after implantation.

In conclusion, both FDP tendon and ACL showed significantly less frictional force than PAT and ACH. In comparison with PAT and ACH, more lubricin was found on the surface and extracellular matrix of the FDP tendon and ACL. The results showed that, compared with ACH and PAT, the FDP tendon had the closest frictional force, compressive moduli, and lubricin expression to the ACL because both are located in an intrasynovial environment. This similarity suggests that the FDP tendon may be a candidate graft for ACL reconstruction. However, since FDP tendons are not available for autograft due to severe morbidity, the application should be considered only as the allograft for ACL reconstruction. Further in vivo studies are needed to prove this concept.

Acknowledgments

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Figure 1.

Sample Preparation. A, Flexor digitorum profundus tendon, volar side (Zone 2), was used as the friction test site. B, Anterior cruciate ligament, posterior medial side (central). C, Achilles tendon, anterior side (central). D, Patellar tendon, posterior side (central).

Figure 2. Friction Testing Device. A, View from overlooking the device. B, Schematic drawing of the device. C, Typical 3 cycles of forward and reverse frictional testing data.

Figure 3. Friction Testing Results. A, Mean of frictional force from cycles 1 through 100. B, Mean of frictional force in cycles 1, 5, 10, and 100. All connected pairs, $P > 0.05$; all unconnected pairs, $P < 0.05$. ACH indicates Achilles tendon; ACL, anterior cruciate ligament; FDP, flexor digitorum profundus tendon; PAT, patellar tendon. Error bars indicate standard deviation.

Figure 4.

Results of Indentation Testing. A, Typical sample of stress-strain curve for flexor digitorum profundus (FDP) tendon, anterior cruciate ligament (ACL), Achilles tendon (ACH), and patellar tendon (PAT). B, The compressive moduli of each group, all paired group $P<0.05$. Error bars indicate standard deviation.

Figure 5.

Histologic Results. Figure shows flexor digitorum profundus tendon (panels A and E, I), anterior cruciate ligament (panels B and F), Achilles tendon (panels C and G), and patellar tendon (panels D and H). Staining of slides A–D, hematoxylin-eosin; E–H, immunohistochemical staining of lubricin. For all slides, original magnification $\times 200$; scale bar, 50 μm .