What Is The Best Candidate Allograft For Acl Reconstruction? An In Vitro Gliding Characteristics And Histological Study In A Canine Model

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Introduction: Anterior cruciate ligament (ACL) reconstruction has become the gold-standard treatment for an ACL rupture, with an estimated of 100,000 ACL reconstructions performed annually in the USA.[1,2] With the improved donor screening, modern procurement and sterilization techniques, the use of allograft for ACL reconstruction have significantly increased.[1] The commonly used allograft included Achilles tendon, bone-patellar tendon-bone, peroneus longus tendon and hamstring tendon. Recent study by Kropf et al.[2] found ACL-PCL contact occurred in 25 % of native knees. After double-bundle ACL reconstruction, contact between the ACL graft and PCL occurred in 75 % of the reconstructed knees.[2] These suggested that there might be abrasion existed between ACL and PCL in part of native knees and ACL reconstructed knees. Several previous studies have compared ACL with the commonly used allografts from tensile properties perspective.[3] To the best of our knowledge, no study has reported about the graft tendons from a frictional perspective, which is an important parameter for ACL functional performance. Therefore, the main objective of this study was to compare the differences of among ACL, flexor digitorum profundus (FDP), Achilles tendon and patellar tendon with frictional force, indentation testing, an alternative way for tendon mechanical evaluation,[4] and histological methods.

Methods: Sample Preparation: 20 hind legs were harvested from 10 adult male mixed breed dogs (weight 21-26 kg), which is euthanized for other IACUC approved studies. After euthanized, the third FDP tendon (N=20), ACL (N=20), the middle bundle of Achilles tendon (N=20) and the central 1/3 patellar tendon (N=20) were harvested immediately. 8 specimens in each group were used for friction testing, 8 for indentation testing and 4 for histology.

Frictional Testing: The friction test device was designed based on previously established methods.[5] Simax glass rod (Friedrich & Dimmock Inc., Millville, NJ, USA) with a diameter of 4 mm was connected in line with an Arcus motor (Arcus Technology, Livermore, CA, USA). One tendon end was connected to a load cell (Transducer Techniques, MDB-10, Temecula, GA, USA); while the other side was connected to a 2N weight through a pulley. The glass rod rotated clockwise with 2 mm/s for 3 turns, and then counterclockwise 2 mm/s for 3 turns, which was defined as a cycle (Fig. 1 A). Based on preliminary experiments, when the total number of cycles reached 100 cycles the frictional force began to stabilize. So 100 cycles was used for the current study. The frictional force was calculated based on previous studies.[4] Data for each cycle was separated into a forward phase and a reverse phase (Fig. 1 B), and the friction was calculated with (F2-F1)/2. A custom MATLAB (MathWorks, Natick, MA, USA) program was developed to automatically calculate the frictional force for each cycle.
**Indentation Test:** The indentation test was performed according to the previously established method.[5] The samples were cut into segment (the same site as that for friction test) with a length of 2 cm. The samples were placed in an aluminum platform with sandpaper to avoid tendon sliding. Bose ElectroForce 3200 test system (Bose Corporation, Eden Prairie, MN, USA) was used for indentation test. A 3 mm flat and non-porous 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.

**Histological Analysis:** The same sites as that for friction test were used for histology. Frozen sections of 10 μm in thickness were used for hematoxylin-eosin or Masson Trichrome staining. Lubricin immunohistochemical staining was performed using 1 μg/mL anti-lubricin monoclonal antibody (MAb) S6.7925.

**Data Analysis:** One-way ANOVA was used and followed by Tukey-Kramer post hoc test. Statistical significance level was set at P<0.05.

**Results:** The frictional force increased with the number of cycles in four groups. The frictional force of the FDP tendon and ACL was significantly lower than patellar tendon and Achilles tendon at the all cycles (p<0.05). However, there is no significant difference between FDP tendon and ACL (Fig. 2 A). The compressive moduli of the FDP tendon, ACL and Achilles tendon was significantly higher than the patellar tendon (P<0.05) (Fig. 2 B). No difference was found in compressive modulus between FDP tendon and ACL. The FDP tendon and ACL displayed a very smooth surface covered by a single layer of epitelenon cells; while patellar tendon and Achilles tendon showed a relatively rough surface covered by a layer of loose paratenon. The collagen in FDP and ACL seemed more dense compared to the patellar and Achilles tendons (Fig. 3 A-D). Lubricin was highly expressed on the surface and extracellular matrix of the FDP tendon and ACL. The paratenon and extracellular matrix of Achilles tendon expressed some lubricin. However, there was only very limited lubricin expression on the surface and extracellular matrix of patellar tendon (Fig. 3 I-M).

**Discussion:** This is the first study to compare their difference in frictional properties, which is an important factor for ACL reconstruction. We found intrasynovial tendon (FDP tendon) and intrasynovial ACL had similar frictional properties, which was superior to extrasynovial tendon (Achilles tendon and patellar tendon). Lubricin expression was also higher than the extrasynovial tendons. The major limitation of the study was that the hamstring tendon, which is a common graft for ACL reconstruction, was not included, since there are no hamstring tendons in canine. However, based on our assumption that hamstring tendon also belongs to extrasynovial tendon, its surface properties may be inferior than either intrasynovial ACL and FDP tendon. This study used canine tissues, which may differ from human.

**Significance:** The FDP tendon has the closest frictional force, compressive moduli, and lubricin expression to the ACL. This similarity indicated that the FDP tendon may be one of the candidate grafts for ACL reconstruction. However, since the FDP tendons are not available for autograft due to severe morbidity, the application should be only considered as the allograft for ACL reconstruction. Further in vivo studies are needed to prove this concept.
Fig. 1 A: Testing apparatus, and B: typical three-cycle motion data.

Fig. 2 A: Frictional results (horizontal bars indicate no significant difference. B: Indentation testing results.

Fig. 3 A: Histology picture (H&E, Masson Trichrome, and Lubricin staining). The ACL (A) and FDP tendon (B) have a very smooth surface, while the Achilles tendon (C) and patellar tendon (D) showed a rough surface covered. The collagen in FDP and ACL seemed more dense compared to the patellar and Achilles tendons (Fig. 3A-D). Lubricin was highly expressed on the surface and extracellular matrix of the FDP tendon and ACL compared to Achilles and patellar tendons (Fig. 3I-M).

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