Mechanical Properties of Canine Patella-Ligament-Tibia Segment

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Objective: To test the ex vivo mechanical properties of canine patella-ligament-tibia (PLT) segment and establish the relationship between donor size and PLT dimensions to the mechanical properties of PLT grafts.
Study Design: Ex vivo mechanical testing study.
Sample Population: Canine PLT segments (n = 21 dogs; 42 PLT).
Methods: Morphometric measurements of PLT segments were taken from computed tomography (CT) images and compared with results obtained using calipers. PLT were tested to failure at a rate of 100% length/s. Mechanical properties and failure mode were recorded.
Results: PLT width and thickness ($P < .001$ for both) measured by calipers were significantly lower than those taken from CT images. Thirty-five (83%) specimens failed by avulsion fracture from the patella, 1 failed mid-ligament, and 6 failed by tibial fracture. Dog weight and PLT length had the strongest Pearson's r value when correlated with load at failure ($r = 0.73$, 0.81, respectively).
Conclusion: Dog weight and PLT length were the best predictors of load at failure. PLT failure load of dogs weighing >25 kg were similar to those reported for the cranial cruciate ligament (CCL) suggesting that the PLT may be a suitable allograft for CCL replacement.

Rupture of the cranial cruciate ligament (CCL) is the most common cause of lameness in dogs,[1] and dog owners in the United States spend more than $1.2 billion annually on veterinary care of this disease,[2] with surgery providing the best short-term prognosis.[3] However, even with surgery, debilitating osteoarthritis progresses in nearly all dogs.[4–6] One possible explanation for this is that none of the currently performed surgical procedures for CCL rupture in dogs reproduce the anatomy or mechanical responsibilities of the intact CCL.

The most commonly performed surgical procedures for canine CCL rupture are extra-articular sutures (e.g., lateral fabellar suture) and tibial osteotomies (e.g., tibial plateau leveling osteotomy, tibial tuberosity advancement).[7] Extra-articular sutures act to limit cranial translation and internal rotation of the tibia with respect to the femur. Limitations to these repairs include suture elongation and yield properties well below the mechanical loads of the normal CCL.[8,9] It has also been suggested that the points of fixation are quasi-isometric at best, and have only been evaluated in 2-dimensions.[10] Tibial osteotomies have been shown in static ex vivo models to reduce cranial motion of the tibia during joint loading at mid-stance but may have no capacity to limit internal rotation (a salient function of the normal CCL) and have a comparatively higher complication rate and financial cost.[11–14] In addition, recent in vivo fluoroscopic imaging studies indicate that cranial tibial translation occurs around the time of paw-strike.[15]

Whereas these procedures all provide reasonable short-term (6-month) success, long-term, they yield a patient that has a 15–20% chance of reoperation and 90–100% chance of arthritis.[16,17] This contrasts sharply with outcome in people where the probability of reoperation is 3–5% and 5-year studies show that <20% of adult anterior cruciate ligament (ACL) surgical patients develop arthritis.[18] Although differences in anatomy and physiology could explain some of these differences, the current intra-articular ACL replacement surgical procedures performed in people may better reproduce the anatomy and mechanical duties of the normal ACL. The most common surgical repairs for people involve arthroscopic placement of intra-articular auto- or allo-grafts at the origin and insertion of the original ligament. These grafts commonly come from the patellar ligament.[19]

Intra-articular grafting for the CCL deficient stifle in dogs has been attempted with several variations of an "over the top" technique using a small portion of the patellar tendon.[20] Intra-articular grafting is currently less commonly performed because of poorer outcomes.[21] Three likely mechanical contributions to failure (biologic contributions to failure may also exist) of this technique include: (1) the patellar ligament autograft (central third of the tendon) is too small and does not possess the necessary mechanical properties[22]; (2) the graft

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was not placed at either the origin or insertion of the original CCL; and (3) fixation of the graft was mechanically weak. CCL allografts have been studied in dogs. Although most dogs still had laxity immediately after surgery, 2 dogs (only 6 were studied in this short-term study) that had stable knees after surgery had resolution of lameness, suggesting that success using an allograft is possible in the dog.[23]

Our purpose was to establish the relationship between donor size, patella-ligament-tibia (PLT) dimensions, and the mechanical properties of PLT segment to assess its mechanical viability as an allograft for intra-articular CCL replacement. We hypothesized that PLT segments from adult dogs have mechanical properties that correlate to the weight of the donor dog.

MATERIALS AND METHODS

Canine stifles (n = 42) were collected from 21 mature dogs (mean ± SD weight, 22.0 ± 11.5 kg; range, 1.5–49.5 kg). Dog maturity was based on dentition; however, specific ages were not available. All specimens were collected within 4 hours of death. For inclusion, each stifle had to be normal (no osteoarthritis, ligament, meniscal, or cartilage lesions) on direct visual examination. The PLT was harvested by dissecting all soft tissues and muscles from the patella, ligament, and tibial tuberosity. The PLT was freed by osteotomy of the proximal third of the tibia. PLT were stored in saline (0.9% NaCl) solution moistened towels, sealed in plastic bags and frozen at −80°C. Specimens were thawed at room temperature for 24 hours before testing.

All PLT specimens were assessed using computed tomography (CT; HiSpeed CT/e GE Medical Systems, Waukesha, WI) and had to be normal (no bone or ligament lesions). Specimens were positioned so the ligament laid flat on the table. Width, thickness, and cross-sectional area of the PLT were taken immediately distal to its attachment to the patella, mid-ligament, and immediately proximal to its tibial attachment using measuring tools in the Kodak viewing program (Kodak PACS Carestream, Rochester, NY). Measurements were performed 3 times by 1 investigator and averaged.

In preparation for mechanical testing, the tibia was potted in a 2-part urethane resin (SmoothCast 300, Smooth-On, Easton, PA) without interfering with the PLT. The proximal segment was secured using a custom built holding fixture that braced the distal aspect of the patella (Fig 1). The holding fixture was designed after pilot work showed that potting of the patella consistently resulted in patella pull-out from the potting material and pinning the patella resulted in patella fracture at the pin site at loads lower than expected.[22]

PLTs were loaded into a materials testing unit (MTS Mini Bionix test frame; MTS Systems Corp., Eden Prairie, MN) and placed under tension at 5 N before measurements. Specimens were under this load for <3 minutes while measurements were taken. Although unlikely at 5 N, creep was not measured during this period. Specimens were oriented so that the bony structures (patella and tibia) were visually aligned coaxial with the actuator with no twist in the ligament. Morphometrics

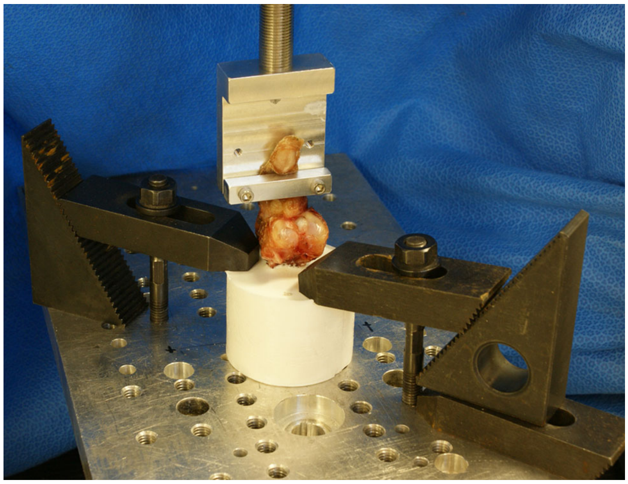

Figure 1 Custom built clamp to brace the distal aspect of the patella.

(length, width, and thickness) of the patella ligament were measured with manual calipers on the cranial surface of the PLT. Width and thickness were measured at mid-ligament. The amount of pressure applied during the measurement process was not controlled beyond visual contact between the caliper and the ligament. PLT length was measured using the caliper by measuring from the attachment of the ligament at the distal end of the patella to the ligament's attachment on the tibia. Cross-sectional area (CSA) was calculated from the caliper measurements on the assumption that the PLT was elliptical on cross-section.

Each specimen was loaded to failure by applying 100% length of the PLT/s.[24,25] Failure was defined as 2 times the original length of the ligament[20–26,27] or a sudden drop on the load/displacement curve. Testing was recorded by computer and video and failure mode determined by direct examination followed by video confirmation. Specimens were included for statistical analysis if failure occurred at the patella, ligament, or tibia and were excluded if failure occurred at the site of potting. Stiffness was calculated from the slope of the load displacement curve (N/mm).

Data Analysis

Statistical analysis was completed using a Student's paired *t*-test when comparing differences in morphometric data based on location of the obtained measurement (proximal vs. middle vs. distal ligament) and method of obtaining the measurement (calipers vs. CT). Pearson's correlation coefficient was calculated to estimate the relationship between body weight, PLT morphometrics, and ultimate load at failure of the PLT. $P < .05$ was defined as statistically significant. Data are presented as a mean ± SD.

RESULTS

All PLT segments were normal on visual inspection and all specimens appeared normal on CT scan. Measurements taken

Table 1 Mean (±SD) CT and Caliper Morphometric Measurements (mm)

	CT Measurements				Calipers
	Proximal Ligament	Mid-Ligament % of Proximal Measurement	Distal Ligament % of Proximal Measurement	Mean	Mid-Ligament (Calipers)
Width	13.01 ± 3.4	92.71	97.63*	12.43 ± 2.8	9.93 ± 2.94
Thickness	2.03 ± 0.67	101.22*	94.21	1.93 ± 0.51	1.70 ± 0.38
CSA	25.43 ± 10.3	95.11	89.16*,**	23.65 ± 10.1	13.47 ± 6.48

*Significantly smaller than the proximal measurement.
**Significantly smaller than the mid-ligament measurement.

by calipers (width, thickness, and CSA) were significantly lower compared with CT measurements ($P < .001$ for all variables). Length was not compared as specimen tension was not standardized for the CT scan. CT measurements revealed that PLT thickness was significantly larger proximally compared to the distal measurement ($P = .04$). Similarly, PLT width measurements were significantly greater proximally compared to the mid-ligament ($P = .0009$). CSA was significantly greater for both the proximal ($P < .001$) and mid-ligament ($P = .023$) compared to the distal ligament (Table 1).

Thirty-five (83%) specimens failed at the attachment of the patellar tendon by avulsion fracture (fragment typically $<5\ mm^2$). One specimen failed mid-ligament and 6 failed by fracture of the proximal tibial diaphysis. All paired limbs had similar (±10%) ultimate failure loads except for PLT segments from the smallest dog (weight, 1.5 kg) where the failure loads were 61 N and 811 N. From video review of tests for this dog, failure load at 61 N was likely from testing error as the specimen was not properly fitted into the testing apparatus; accordingly, these data were not included in statistical analysis.

The lowest failure load was 811 N from a dog weighing 1.5 kg and the largest was 3451 N from a dog weighing 45.9 kg. Failure load had its strongest correlation (r = 0.81; Fig 2) with patellar ligament length followed by dog weight (r = 0.73; Fig 3). Ligament width, CSA, and thickness had lower correlations with load at failure (r = 0.51, r = 0.38, r = 0.37, r = 0.29, respectively). All but 1 PLT from a dog with a body weight >25 kg had a load at failure >1750 N. Stiffness of the PLT segments for dogs >25 kg was 215 N/mm.

DISCUSSION

We found that adult dog PLT segments have mechanical properties that correlate with dog weight; however, given the correlation between failure and body weight (r = 0.73), other factors must contribute to the variation in PLT segment mechanical performance. The stage of skeletal maturity in dogs has been reported as influencing the mechanical properties of the patellar ligament.[22] Because of the source for the dogs, specific ages were not available, so maturity was assessed based on dentition. It would have been ideal to see if aging had an influence on PLT mechanical performance as it does for the canine CCL.[28] Breed could also influence PLT mechanical properties similar to the CCL.[26,29] The activity level of the donor dog before euthanasia could influence PLT strength; however, a clear difference between the CCL and PLT is the high prevalence and breed differences of naturally occurring CCL disease in dogs. These breed differences do not appear to exist for naturally occurring PLT tears. We were somewhat surprised that CSA did not correlate with PLT mechanical properties, because we expected that a greater CSA would have more fibers and provide greater strength. From this one could conclude that a simple measurement of CSA may not be an accurate estimate of the number or size of the fibers in the PLT. We did not perform histologic evaluation of PLT to confirm or refute this relationship.

One of our objectives was to determine if PLT segments from adult dogs would have ultimate failure loads sufficiently high for use as an allograft in CCL deficient dogs. Because we did not test CCL mechanical properties, this objective was geared toward comparing our findings to reported data. Few studies of CCL mechanical properties have correlated ultimate failure load with body weight.[28,30–32] Healthy Beagle (no weights reported) CCL has a tensile strength of 210–556 N.[27] For mixed breed dogs (no weights reported) failure load has been reported as 1151 N[24] and 1656 N.[33] Figgie et al.[34] studying Beagles showed that stifle testing angle also affects ultimate load (0° = 1181 N, 45° = 454 N and 90° = 428 N). Normal Rottweiler (mean weight, 42 kg) CCL tested in 130° of flexion and loaded in a cranial direction had an ultimate load of 2130 N and failed at 1738 N when tested along the axis of the ligament. Greyhound (mean weight, 31 kg) CCL tested in 130° of flexion and loaded in a cranial direction had an ultimate load of 1799 N and failed at 1781 N when tested along the axis of the ligament.[26] For mixed breed dogs, ligament failure occurred at

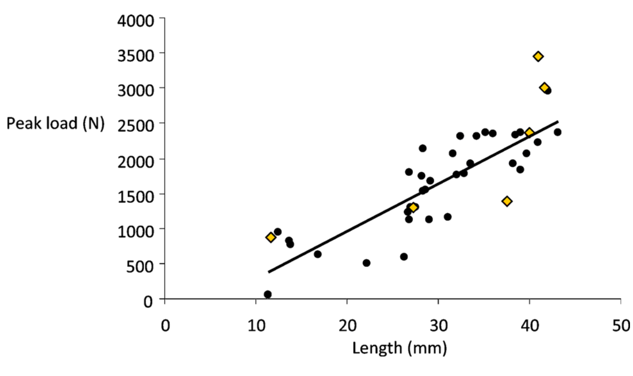

Figure 2 Relationship between peak load (N) and ligament length (mm). r = 0.81. Trendline equation: y = 68.276x − 410.84.

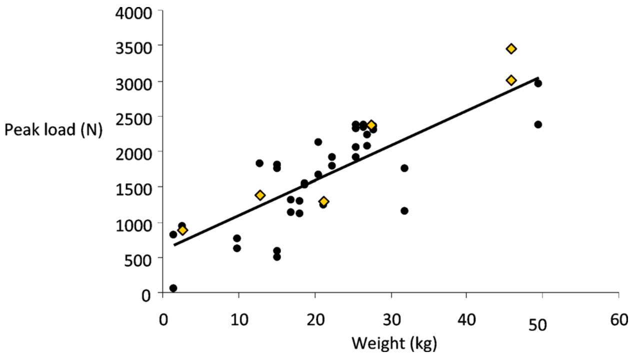

Figure 3 Relationship between peak load (N) and donor weight (kg). r = 0.73. Trendline equation: y = 49.389x + 591.07.

590 N (average weight, 20 kg)[35] and 687 N (average weight, 16.7 kg).[24] Foxhounds (average weight, 26.8 kg) had CCL failure at 1129 N.[36] Johnson et al.[20] reported an average failure load of 1469 N for dogs weighing 14–25 kg. The difference between studies is likely because of specimen preparation (frozen, fresh, sterilized), testing apparatus (potting material, clamps), testing angle, displacement rate, and breed differences.

In our study, all but 1 dog with a body weight >25 kg had a failure load <1750 N. Although we were unable to perform direct statistical comparison, this finding compared with the cited literature suggests that the PLT has an ultimate failure load that is similar to CCL failure loads in dogs weighing >25 kg. For example, a PLT from a 30 kg dog should have similar strength to the CCL in a 30 kg recipient. A second comparison can be made between stiffness of the 2 ligaments. In dogs >25 kg, PLT stiffness was 215 N/mm compared with 224 N/mm for the CCL.[26] The relationship between dog weight and ultimate strength may be useful if one is deciding what PLT allograft size should be used for an individual patient.

During a walk, the CCL resists 50 N of force and at vigorous play, loads are 400–600 N.[37] Evans et al.[38] have shown at a walk, that vertical ground reaction forces are ~40% of dog weight (e.g., for a 40-kg dog, ground reaction force is ~160 N). These estimates suggest a replacement graft for a ruptured CCL may not have to equal the strength of the original CCL and grafts of less strength may be adequate.

Use of the central third of the patella ligament similar to a technique used in people has been suggested, but the biomechanical strength is less than the normal CCL and comes with morbidity at the donor site.[39,40] Haut et al. reported an average failure load of 2149 N for the PLT in medium and large breed dogs (18.3–35.1 kg) aged 0.5–15 years.[21] Specimen storage, apparatus design, and displacement rate was similar between studies, and we had very similar findings. For specimens in our study in that weight range (18.3–35.1 kg), mean failure load was 2107 N. The small difference may be because of differences in distribution among the weight bracket, breeds studied, and dog age.

There are clear differences in the length of the patellar tendon and the CCL. However, if one were considering using the PLT segment for CCL replacement, the functional length of the PLT graft could be made similar when accounting for twisting of the graft during placement[41] and the location of the femoral and tibial fixation points (e.g., interference screws). Differences in other dimensions (width, thickness) may also have to be accounted for before the PLT could be considered as a replacement for the CCL.

CT was used to take morphometric measurements and screen the PLT for abnormalities. Although MRI is the gold standard for assessing ligaments, it has been shown that the CT provides adequate detail and is likely a reasonable screening test for detecting ligament abnormalities.[42] Measurements were also taken manually using calipers, because CT may not be available or feasible when collecting and/or implanting grafts in a clinical setting. Use of calipers consistently provided lower measurements when compared with CT measurements. This is consistent with previous reports that showed calipers underestimated CT measures by 15–40%.[43] One limitation in making a direct comparison is that tendons were measured by CT in an unloaded conditions and by calipers in a loaded (5 N) condition.

The width, thickness, and CSA of the ligament varied based on the location. This is also seen in the CCL[26] although it has not been described for the canine patella ligament. This could be important if width or thickness dimensions were used to help choose an appropriate graft.

One possible limitation of our study is that most specimens failed by avulsion fracture from the patella. These fractures did not occur near the site where the PLT was in contact with the holding fixture suggesting that this may be the mechanically weakest area of the PLT. Our findings are similar to those reported by Haut et al. where 16/27 specimens failed by avulsion of the patella.[19] When evaluating the 2 studies, one could conclude that this mechanism of failure is consistent in skeletally immature and mature dogs. Other limitations include that the mechanical testing of the PLT segment did not include cyclic loading or behavior under different loading rates.

Our goal was not only to test the patellar ligament but also describe certain mechanical properties of the PLT. We found that PLT appears to have mechanical properties that would make it suitable as an allograft replacement for the CCL; however, before considering clinical allograft replacement additional scientific evidence is required. Multiple other studies have looked into bone-ligament-bone allografts for canine CCL replacement grafts. Vasseur et al. used CCL allografts in 6 dogs that were sacrificed 9 months later. All dogs had stifle instability, 2 joints had no identifiable CCL allograft visible, the allografts appeared immunogenic and had only 14% the strength of the intact CCL.[44] Another study looking at CCL allograft host incorporation showed that by 4 weeks there was no longer donor DNA detectable, and the amount of collagen and collagen structure was different from the control CCL. The findings were not correlated to biomechanical strength.[45] Although these studies are not encouraging for allograft survival in the intra-articular environment, many factors have been identified that affect graft healing and incorporation including but not limited to location of graft placement, graft length in the bone tunnel and the graft versus tunnel diameter ratio, method of graft fixation, graft tension, patient rehabilitation, and immune response.[46] In the aforementioned papers, many of these factors were not separated and are the main reason why a stepwise process will have to be undertaken before a PLT allograft can be considered for clinical use. For example, a logical next step would be to test the strength of fixation of an allograft PLT to ensure that an implanted graft still demonstrates adequate mechanical properties to be considered for future investigation. Our results can be translated to future work because we now have a better understanding that the ultimate failure load of a PLT graft can be estimated based on the weight of the donor and the length of the PLT graft.

DISCLOSURE

The authors report no financial or other conflicts related to this report.

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