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In Vivo Evaluation of Intra-Articular Protection in a Novel Model of Canine Cranial Cruciate Ligament Mid-Substance Elongation Injury

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Abstract

Objectives—To evaluate the effects of intra-articular protection (IAP) on the canine cranial cruciate ligament (CrCL) and stifle in a CrCL midsubstance elongation injury model.

Study Design—Experimental longitudinal cohort study.

Animals—Skeletally mature female mixed breed hounds (n = 12; mean ± SEM weight, 25.6 ± 0.7 kg).

Methods—After CrCL elongation in 1 stifle of each dog, IAP was applied in 6 joints. In vivo assessment included radiographs, cranial–caudal joint translation, gait analysis, and synovial fluid levels of 3B3(–) (proteoglycan epitope) and C2C (collagen II neoepitope) up to 12 weeks after surgery. Joint translation and rotation were quantified at necropsy. CrCL midsubstance length was determined before and after elongation and at necropsy. CrCLs were subjectively assessed with light microscopy. Comparisons were made between stifles containing elongated CrCLs with and without IAP and unoperated controls.

Results—Four weeks after surgery, ground reaction forces were significantly decreased in operated limbs. Absolute C2C levels were significantly elevated in operated stifles 4 weeks post-surgery. C2C and 3B3(–) levels normalized to total protein were significantly elevated in IAP + stifles 8 weeks after surgery. Protected CrCLs appeared to have decreased granulation tissue and better collagen fiber alignment.

Conclusions—IAP has negligible effects on the canine stifle based on the response variables evaluated in this 12-week study. Protection of elongated CrCLs may promote reduced, organized scar formation.

Clinical Relevance—These results support the healing capacity of the canine CrCL midsubstance following elongation injury and IAP application to potentially reduce cicatrix formation in elongated CrCLs.

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INTRODUCTION

Elongation injuries of the anterior cruciate ligament (ACL; analogous to the cranial cruciate ligament [CrCL] in the veterinary patient) account for 20–30% of all human knee injuries.¹ The ACL is not completely disrupted in as many as 85% of such injuries, but permanently lengthened because of collagen fiber elongation with or without evidence of partial gross disruption.^{1–3} Reports of the functional outcome of ACL sprains varies widely, but knees that appear stable initially can progress to symptomatic ACL laxity with conservative treatment.^{3,4} There is an abundance of information regarding the relative contributions of specific ACL⁵ and CrCL^{6,7} components to joint stability; and even minimal compromise of ligament integrity results in an unstable joint.⁸ The process of ligamentous healing is well described; and it has been shown that some physiologic stresses on the damaged tissue are necessary for best recovery of a functional structure.⁹ Altered joint kinematics subsequent to compromise of ACL function contribute to degenerative joint changes and likely promote progressive ACL deterioration in some cases.^{8,10–13} Reduction of physiologic stresses by protection of elongated ACLs through the healing stages may facilitate natural restoration of the structures.

Synthetic intra-articular protection (IAP) of biological ACL reconstructions was introduced by Kennedy et al in 1980.¹⁴ The ligament augmentation device (LAD) is designed to protect biological ACL graft reconstructions through the process of ligamentization when graft strength wanes.¹⁵ It effectively shares the physiologic load with the graft, protecting it from potentially disruptive forces without complete stress protection.¹⁶ Many techniques and materials for intra-articular application in the knee have been described subsequent to LAD introduction including protection of primary repairs of acute ACL tears.^{17,18} Based on published studies, superior clinical results are achieved in both canine^{19,20} and human^{17,18} patients with augmented repairs.

The dog is an accepted animal model for study of ACL injury, healing, and reconstruction.^{21–24} To our knowledge, a canine midsubstance CrCL elongation injury model with which to study methods to facilitate ligament healing or shortening has not been described. Our 2 objectives were to develop a novel canine model of midsubstance CrCL injury and to evaluate the effects of IAP on the CrCL and stifle. We hypothesized that IAP would facilitate CrCL healing and limit degenerative articular changes.

MATERIALS AND METHODS

Study Design

After CrCL elongation, a randomized block designed was used to assign 1 stifle of 12 skeletally mature female mixed breed hounds (mean $\pm$ SEM weight, 25.6 ± 0.7 kg) to receive (IAP +) or not (IAP–). Contra-lateral limbs served as unoperated controls (IAP + C, contra-lateral limb to an IAP + limb; IAP–C, contra-lateral limb to an IAP– limb).

Treatment group CrCLs were elongated by ~25% using a specifically designed instrument and a standard open surgical approach. IAP was applied in stifles of dogs in the IAP + group before closure. In vivo assessment variables included manual stifle examination, radiographs, instrumented cranial–caudal (Cr–Ca) translation quantification, kinetic analysis, and stifle synovial fluid levels of 3B3(–) (proteoglycan epitope) and C2C (collagen type II neoepitope). Dogs were euthanatized 12 weeks after surgery. Cr–Ca translation and medial–lateral (M–L) rotation were objectively quantified in situ immediately after death, with and without an intact joint capsule. CrCL midsubstance length was determined before and after elongation, and immediately after death. All CrCLs were subjectively assessed with light microscopy. Comparisons were made between stifles containing elongated CrCLs

with (IAP +) or without (IAP-) IAP and unoperated controls of each group (IAP +C and IAP-C).

Surgical Procedure

Dogs were premedicated with acepromazine (0.10 mg/kg subcutaneously) and butorphanol (0.20 mg/kg subcutaneously). After 20 minutes, thiopental (5 mg/kg, intravenously [IV]) was administered for anesthetic induction, then dogs were intubated and maintained on halothane in oxygen administered with a semi-closed circle system. Cephazolin (20 mg/kg IV) was administered preoperatively. Stifles were examined for range of motion, swelling, temperature, crepitus, varus-valgus, patellar tracking, and presence of drawer sign. The designated stifle of each dog was aseptically prepared for surgery.

A 4.0 cm long, medial parapatellar arthrotomy was performed on each stifle. After lateral patellar luxation, the infrapatellar fat pad was gently manipulated to allow complete joint and CrCL examination. Single-interrupted sutures (2-0 silk) were placed ~5 mm apart in the CrCL midsubstance with the distal most suture just proximal to the intermeniscal ligament. A bar type tension gauge (McMaster-Carr, Chicago, IL) was used to apply 2.5 N of tension to the distal aspect of the CrCL. The distance between the distal edge of the proximal suture and the proximal edge of the distal suture was measured 3 times with a venier caliper (McMaster-Carr) during force application to determine the pre-elongation length (PrE). All measurements were performed with the stifle in 135° of flexion. The mean of the 3 measurements was used for all analyses.

A specially designed device was used to stretch each CrCL midsubstance (Fig 1). The device had a hollow cylinder (7.5 mm inner diameter; 10 mm outer diameter) with a 2.5-mm-wide textured (80 grit) rim. Within the cylinder was a removable 10-gauge stainless-steel hook (5 mm diameter, 2 mm depth) attached to a retractable ratchet system that locked in place every 1.5 mm. To effect tissue stretch, the hook was placed beneath each CrCL approximately equidistantly between sutures and retracted until it was entirely inside the cylinder. The textured surface of the cylinder was then engaged with the CrCL surface by pressing the device firmly against the CrCL. This maneuver was to prevent tissue slippage during hook retraction and limit elongated tissue to that within the cylinder. With the stifle held in 135° of flexion, the hook was retracted at a rate of 0.4–0.5 mm/s to a displacement of ~3 mm which was held for 1 minute then released (Fig 2). The technique of CrCL elongation was established with preliminary in vitro work and based on reported maximum canine CrCL strains.²⁵ After stretching, the post-elongation length (PoE) was determined identically to the PrE. The percent elongation (PE) was calculated as $PE = [ABS(PrE - PoE) / PrE] \times 100$.

Stifles without IAP were lavaged with normal saline (0.9% NaCl) solution and sutured closed. In IAP + stifles, the IAP was anchored in the tibia with the Ultrafix Minimite Suture Anchor System™ (Linvatec Corporation, Largo, FL) according to the manufacturer's instructions (Fig 3). Briefly, a bone tunnel (2.4 mm diameter, 14 mm depth) was created in the proximal tibial surface just cranial to the intermeniscal ligament and at the midpoint (medial to lateral) of the CrCL insertion. Two strands of size 2 braided polyester (Ethibond Excel, Ethicon Inc., Somerville, NJ) were threaded through the eyelet of the suture anchor to their midpoints to give a total of 4 intra-articular strands. The anchor was deployed in the bone tunnel. A 3 cm incision through skin and subcutaneous tissue was centered over the lateral fabella at a 45° angle to the long axis of the femur. Using a drill guide, a 2.4 mm bone tunnel was created in the lateral aspect of the femoral condyle from medial to lateral just proximal and centered on the origin of the CrCL at an angle of approximately 45° from the sagittal axis of the femur. The stifle was positioned in 135° of flexion; sutures were passed beneath the intermeniscal ligament, along the cranial aspect of the CrCL, and through the

bone tunnel (Fig 4). Gentle tension was applied until there was no drawer motion, and the suture strands were secured to the femur with a belt-buckle technique just proximal and cranial to the lateral fabella with three 7×7 mm bone staples using a powered metaphyseal stapler (Stapilizer, 3M, St. Paul, MN).²⁶ Long suture tags were removed.

Butorphanol tartrate (0.2 mg/kg IV) was administered once during anesthetic recovery and then intramuscularly every 4 hours for 24 hours as needed for postoperative analgesia. Postoperatively, all dogs were administered cephazolin 6 hours after the first dose (20 mg/kg subcutaneously). Each dog was administered etodolac (10 mg/kg orally every 24 hours) and enrofloxacin (10 mg/kg orally every 24 hours) beginning 24 hours after, and continuing for 7 days after surgery. Dogs were confined to 4×6 ft² runs for the study.

Kinetic Gait Analysis

Kinetic gait analysis was performed preoperatively and 4, 8, and 12 weeks after surgery as previously reported.^{26,27} A total of 3 handlers experienced in canine force platform gait analysis participated equally in the gait trials as variance attributable to trained handlers is negligible.²⁸ A trial was considered successful if a fore paw contacted the force plate followed by the ipsilateral hind paw at a velocity of 1.70–2.60 m/s and acceleration of -0.9 – 0.9 m/s²; ranges that included a comfortable trotting pace for each dog in the study. Measured forces included peak vertical force (PF) and vertical impulse area (IMP), and all recorded forces were normalized to body weight. The first 3 valid trials were recorded at each time point.

In Vivo Stifle Translation

Standard Cr–Ca and M–L radiographic projections were taken preoperatively and 4, 8, and 12 weeks after surgery. Radiographs were evaluated for signs of osteoarthritis. Additionally, lateral stress radiographs were performed for quantification of in vivo Cr–Ca stifle translation using a device and methodology described previously.²⁹ All radiographs were performed with the animals anesthetized as described above, and synovial fluid was collected aseptically from each stifle before recovery. Duration of general anesthesia was 30–45 minutes.

The total (TTT), cranial (CrTT), and caudal (CaTT) tibial translation was measured in each stifle. All tibial translations were normalized to the size of the tibia by dividing the translated distance by tibial width, measured just distal to the tibial tuberosity perpendicular to the long axis of the tibia, and multiplying by 100.²⁹

Synovial Fluid Biomarkers

Synovial fluid (0.5–2 mL) was centrifuged at 200 g. for 15 minutes, aliquoted, and frozen at -80°C . Aliquots were thawed at room temperature just before analysis. 3B3(–) was assayed by enzyme-linked immunosorbent assay (ELISA) according to published methods.³⁰ C2C was quantified using C2C ELISA kits (IBEX pharmaceuticals Inc., Montreal, Quebec, Canada) according to the manufacturer's instructions. Total protein was quantified using a temperature compensated hand-held refractometer (Reichert Analytical Instruments, Depew, NY). All assays and evaluations were performed in duplicate. For purposes of analysis, absolute synovial fluid biomarker levels were considered in addition to levels normalized to baseline (absolute/preoperative) and total protein (total protein/absolute).

In Situ Mechanical Testing

In situ stifle Cr–Ca translation and M–L rotation tests were performed immediately after death. Each translation test was followed by a rotation test. Testing was performed first with, and then without, intact joint capsule. Specimen preparation and mechanical testing was

performed as detailed previously.²⁷ Upon conclusion of mechanical testing, any IAP sutures present were cut, and the final elongation length (FE) was measured as before in surgically treated stifles. The final percent elongation (PEF) was calculated as $PEF = [ABS(PrE - FE) / PrE] \times 100$. In vitro total, cranial, and caudal tibial translation and total, medial and lateral rotation were normalized to tibial width.²⁹

Histologic Sample Preparation

CrCLs were collected by sharp transection at each origin and insertion. Tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sagittal sections (5 μ m) were processed for histologic staining with Masson's trichrome, and hematoxylin and eosin. Nine sections from each CrCL were collected from the approximate center of each ligament. Fibrocyte morphology and collagen fiber architecture were subjectively evaluated with light microscopy. CrCL bony insertions were not assessed in this study. To avoid potential bias, evaluators were unaware of treatment group information.

Statistical Analysis

All response variables were considered continuous and tested for normality using the Shapiro–Wilk statistic with the null hypothesis of normality rejected at $P \leq .05$. A multivariate ANOVA was used to evaluate fixed effects of group (IAP + versus IAP–) and CrCL treatment (elongation versus none; IAP + and IAP– versus IAP + C and IAP–C) on the collinear response variables. The random variance of dog nested within group was included and used as the error term for effect of group. When responses were measured over time, the fixed effect of time and its interactions were added to the model and a repeated measures, multivariate analysis of variance was performed. Wilk's λ was evaluated first for the interaction effects and then for the main effects. When Wilk's λ was significant at $P \leq .05$, the null hypothesis of no overall multivariate effect was rejected and univariate analysis of variance with a significant F statistic ($P \leq .05$) were identified. For significant univariate analyses, if there were significant interactions of group $\times$ CrCL treatment and/or time, then predetermined comparisons within or between groups across treatments (and time) were performed using Tukey's adjustment to maintain type I error at 0.05. The CrCL midsubstance lengths and elongation percentages (PrE, PE, and PEF) were compared with paired or unpaired t-tests within and among CrCL treatments, respectively. Velocity and acceleration among treatment groups at each time point and among time points were evaluated with unpaired t-tests and repeated measures ANOVA, respectively. Statistical analyses were performed with commercially available software programs (SAS Release 6.12, SAS Institute, Cary, NC, [PROC GLM, PROC UNIVARIATE] or GraphPad Prism v4.0, GraphPad Software Inc., San Diego, CA [t-tests, repeated measures ANOVA]). Results were considered statistically significant at $P \leq .05$. For purposes of this report, significance indicates statistical significance. All results are reported as mean $\pm$ SEM.

RESULTS

CrCL Elongation

Elongated CrCLs became somewhat translucent after stretching and appeared grossly flaccid, but there was no gross evidence of disruption or laceration after elongation or on necropsy examination. Intra-articular changes were limited to mild to moderate chondrophytosis along the abaxial surfaces of each trochlear ridge in all treated stifles 12 weeks after treatment. Untreated stifles were grossly normal with intact CrCLs. PrE was 5.42 ± 0.01 and 5.48 ± 0.22 mm; PE was $24.91 \pm 4.08\%$ and $25.92 \pm 2.92\%$; and FE was $23.66 \pm 3.94\%$ and $24.66 \pm 4.23\%$ in IAP– and IAP + stifles, respectively. PrE was not significantly different between treatments, and PE and FE were not significantly different between or from each other within treatments.

Kinetic Gait Analysis

The velocity and acceleration of all gait trials were 2.10 ± 0.02 m/s and -0.03 ± 0.03 m/s/s, respectively. There were no significant differences in velocity or acceleration among treatments or time points. There were significant overall multivariate effects on both PF and IMP ($P < .05$). Univariate analysis of PF showed a significant interaction of CrCL treatment $\times$ time ($P < .001$) and CrCL treatment $\times$ group ($P < .001$). The PF of limbs with control stifles (IAP +C and IAP -C) was significantly greater than those with operated stifles (IAP + and IAP -) 4 weeks after surgery (Fig 5A). The overall PF for limbs with IAP +C stifles was significantly greater than limbs with IAP - stifles (Fig 5B). Though not significant, the difference in mean PF between limbs with IAP - and IAP -C stifles (1.5%) was about half that between limbs with IAP + and IAP +C stifles (2.8%). Univariate analysis of IMP showed a significant interaction of CrCL treatment $\times$ time ($P < .001$). The IMP for control stifles 4 weeks after surgery was greater than operated stifles 4 and 12 weeks after surgery and greater than control stifles 8 weeks after surgery (Fig 5C). There was a significant but poor correlation between PF and IMP ($r = 0.57$, $P < .001$).

In Vivo Stifle Translation

All preoperative radiographs were within normal limits and postoperative IAP -C and IAP +C radiographs were normal for the duration of the study. Radiographic changes in treated stifles were limited to mild to moderate stifle effusion from 4 to 12 weeks postoperatively. Distinct differences in effusion between treatment groups and among time points were not evident. There was no significant overall multivariate effect on any of the in vivo translation response variables over the course of the study (Table 1).

Synovial Fluid Biomarkers

There were significant overall multivariate effects on absolute synovial fluid levels of C2C and 3B3(-). Univariate analysis of 3B3(-) was not significant but analysis of C2C levels showed a significant interaction of CrCL treatment $\times$ time ($P = .003$; Fig 6). C2C levels in treated stifles (IAP + and IAP -) 4 weeks after surgery were significantly greater than unoperated control stifles (IAP +C and IAP -C) preoperatively, 8 and 12 weeks after surgery and than treated stifles 8 weeks after surgery (Fig 6A). There was a poor correlation between 3B3(-) and C2C absolute levels ($r = -0.05$, $P = .71$). There were also significant overall multivariate effects on synovial fluid levels of C2C normalized to total protein concentration. Univariate analysis showed a significant interaction of group $\times$ CrCL treatment $\times$ time ($P < .001$; Fig 6B). C2C levels in IAP + stifles 8 weeks after surgery were significantly greater than preoperative levels in IAP +, IAP +C, IAP -, and IAP -C stifles and than those in IAP - and IAP +C stifles 4 weeks after surgery. Univariate analysis of 3B3(-) normalized to total protein showed a significant interaction of group $\times$ CrCL treatment $\times$ time ($P < .001$; Fig 6C). Levels in IAP + stifles 8 weeks after surgery were significantly greater than preoperative levels in IAP +, IAP +C, and IAP -C stifles. There was a significant but poor correlation between 3B3(-) and C2C normalized to total protein ($r = 0.37$, $P = .007$). Though not significant, 3B3(-) levels normalized to total protein were consistently highest in the IAP + stifles beginning 4 weeks after surgery to study end. There were no significant overall multivariate effects on C2C or 3B3(-) normalized to baseline.

In Situ Mechanical Testing

There were no significant multivariate effects on in situ mechanical testing response variables (Table 2).

Histology

The cranial and caudal surfaces of all elongated CrCLs were covered by synovium. Both normal and elongated tissue was apparent in each section. Normal ligament, apparent at the proximal and distal ends of each section, consisted of closely packed collagen fibers with characteristic wave forms and fibroblasts sparsely scattered between them. Defects resulting from collagen fiber disruption were apparent in the mid-substance of all elongated CrCLs. Active tissue repair was evidenced by the presence of amorphous granulation tissue and numerous plump fibroblasts within the defects (Figs 7A, B). Disrupted collagen fibers were interspersed with non-disrupted fibers across and along the length of the CrCL mid-substance. Based on subjective evaluation by the blinded observers, defects were smaller and the ends of disrupted collagen fibers better aligned by loosely woven repair tissue in the IAP protected CrCLs (Fig 7A) versus the unprotected CrCLs (Fig 7B). Because of differences in collagen fiber disruption between sections as well as fixation and sectioning artifacts, quantification of granulation tissue and alignment of disrupted collagen fibers was not possible.

DISCUSSION

A novel canine CrCL midsubstance elongation model was used to evaluate the effects of IAP on the stifle and elongated CrCL. Published information indicates that the canine CrCL ruptures at ~36% strain.²⁵ The device we used applied a strain of 25% because this resulted in consistent elongation and disruption of midsubstance microstructure without complete ligamentous rupture. CrCLs consistently appeared loose after stretch, and elongation was maintained over the 12 week study. Collagen fiber disruption was evident in both treatment groups 12 weeks after injury, but, based on subjective assessment, fibers were better aligned and granulation tissue reduced in the IAP + treatment group. These findings are consistent with current knowledge that clinical ACL,¹⁴⁻¹⁷ or experimental CrCL^{17-20,31-32} disruptions can heal after primary repair without IAP, and the natural repair process is optimized by IAP attenuation of physiologic stresses. Further studies are necessary to assess potential detrimental effects of long term IAP stress protection on canine CrCL function and mechanical properties.⁹

A midsubstance CrCL elongation of 25% over the 5 mm span between sutures corresponds to an average length increase of 1.3 mm. Whereas this length change is likely important to the dynamic joint stabilizing function of the CrCL,^{6,7} the ability to detect resulting increases in tibial translation or rotation in vivo is difficult. Detection of minute human ACL,³³⁻³⁵ and canine CrCL^{29,33-35} elongation by tibial translation has been and continues to be an orthopedic challenge. It is not surprising that the normalized in vivo tibial translation values determined over the course of the study were consistent with those previously reported for canine stifles with intact CrCLs given the relatively small increase in CrCL length and the presence of surrounding soft tissues and joint structures.²⁹ Stifle stability changes were also not detected in situ. For purposes of comparison to earlier studies, in situ mechanical testing was performed as done previously with all intra- and peri-articular ligamentous and tendinous structures intact with the exception of the patellar tendon. Had all such structures been removed, or increased loads applied, differences in translation and rotation between treatment groups may have been detectable. In addition, partial transection of the CrCL may have increased abnormal tibial motion to levels detectable with the methodology used. The model we developed was one of CrCL elongation without gross disruption, so partial CrCL transection was not performed.

The use of ground reaction forces for objective characterization of canine gait is well established.^{36,37} The significant gait alterations evident in both PF and IMP 4 weeks after surgery are likely a surgical effect given that there were no differences between stifles with

or without IAP, and differences between operated and control limbs subsequently resolved. Of interest is the overall smaller difference in PF between control and treatment limbs in the IAP + group versus the IAP- group. Though the difference was not significant, it may be suggestive of greater symmetry between hindlimbs in IAP + dogs. Further work is necessary to evaluate this effect.

Synovial fluid biomarkers are sensitive indicators of joint degeneration in the dog, and 2 such markers established for detection of early cartilage changes are 3B3(-) and C2C. 30·38·39 3B3(-) is an epitope in native chondroitin sulfate on aggrecan detected by the IgM monoclonal antibody 3B3 in the absence of chondroitinase ABC pre-treatment; C2C is a neopeptide created by collagenase cleavage of type II collagen that is recognized by the C2C antibody (formerly called the Col 2 3/4C long antibody).38·40 The elevation in C2C synovial fluid levels in operated stifles 4 weeks after surgery may be the result of inflammation secondary to surgical trauma. Elevation of synovial fluid C2C levels because of joint instability or articular cartilage abrasion from IAP sutures is less likely as there were no differences between stifles with or without IAP 4 weeks after surgery, and the differences between operated and unoperated stifles resolved by 8 weeks after surgery. 3B3(-) and C2C elevations 8 weeks postoperatively may reflect the return to normal limb use after a period of natural protection associated with surgical discomfort. This is supported by the gait kinetic data described above. Though not significant, protein normalized levels of 3B3(-) were consistently elevated in stifles with IAP after surgical application, potentially because of low grade, chronic irritation or cartilage abrasion by the IAP sutures as has been previously reported in human knees.41 As indicated above with respect to the CrCL, a study of longer duration is necessary to assess long term IAP effects on overall joint health. The overall effect of IAP on articular cartilage was minimal in this investigation based on the findings that neat synovial fluid samples had minimal statistically significant changes and levels normalized to baseline did not reveal significant differences between treated and untreated stifles.

Canine CrCL injury models have been the basis for a number of studies including IAP of CrCL graft reconstruction.21·23·24·27·42 Partial or complete CrCL transection is reported in published canine CrCL injury models.27·43 Canine CrCL laxity without injury has been produced by surgical elevation of the CrCL tibial insertion.44 A rabbit CrCL elongation injury description includes partial CrCL transaction.2 Rabbit CrCL subfailure injury has also been effected in vitro by tensile load application to the femur–CrCL–tibia complex.8 To our knowledge, there are no reports of an in vivo canine CrCL midsubstance elongation model.

Difficulty in assessing the microstructural integrity of a visibly intact CrCL is well known. The human ACL can appear macroscopically intact with widespread disruption of collagen fibrils.45 It is likely that the CrCL elongation in our study was permanent as indicated by maintenance of the elongation over the 12 week period. The length of the healed ligament is likely determined by initial trauma, quality of natural repair, and scar maturation and contraction2, but it is not possible to predict this information based on our results. It is possible that microscopic failure similar to that produced in this study may be a cause of clinical instability and progressive ligamentous structural failure.3 The model described here may be useful for studies designed to advance detection and treatment of comparable injuries and to inhibit potential ligamentous deterioration.

A novel model of CrCL midsubstance elongation injury was used to evaluate the effects of IAP on the stifle and elongated CrCL from the microscopic to the macroscopic level in this in vivo study. Based on our results, we find that there were negligible effects of the IAP we applied on the stifle. Based on subjective assessment, protection of elongated CrCLs appears to promote reduced and more organized scar formation compared with unprotected CrCLs

with comparable damage. These results support the healing capacity of the CrCL midsubstance after elongation injury and use of IAP may reduce cicatrix formation in elongated CrCLs. Further research is necessary to establish the long-term effects of IAP on the CrCL and stifle joint.

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Fig 1.

Photograph of the ligament stretching device in use to elongate a canine cranial cruciate ligament (CrCL) through a medial parapatellar arthrotomy. The retractable hook is in place beneath the CrCL and the cylinder rim is pressed against the CrCL surface to limited elongated tissue to that within the cylinder. The patella (P) is luxated laterally and the medial aspect of the femoral condyle (MC) is to the right of the device.

Fig 2. Canine cranial cruciate ligament (CrCL) elongated by ~25%. Sutures to measure CrCL midsubstance length are evident (arrows). The caudal cruciate ligament (PC) is right of the CrCL and the lateral aspect of the femoral condyle (LC) is left.

Fig 3.
Linvatec Ultrafix Minimite Suture Anchor Guntm with suture threaded through the anchor.
A magnified view of the suture anchor is pictured in the inset.

Fig 4.

Intraoperative photograph of synthetic intra-articular protection (IAP) of an elongated canine cranial cruciate ligament (CrCL). The IAP consisted of 4 strands of 2 polyester suture (Ethibond) fixed to the proximal tibia just cranial to the intermeniscal ligament (IML) with a suture anchor (arrow). The strands were passed along the cranial aspect of the CrCL to its origin (arrow head) and then through the lateral aspect of the femoral condyle (LC). The strands were stapled to the femur just caudal to the tunnel with bone staples. The caudal cruciate ligament (PC) is to the right of the suture strands.

Fig 5.

Peak vertical force (A–B) and vertical impulse area (C) of control (IAP–C and IAP +C) and operated limbs (IAP + and IAP–). Overall differences in PF between treatment groups are illustrated in graph B. Columns with different letters within each graph are significantly different ($P < .05$). Values are expressed as mean $\pm$ SEM. IAP, intra-articular protection.

Fig 6.

Synovial fluid levels of C2C (A), total protein/C2C (B) and total protein/3B3(-) (C) in control (IAP-C and IAP +C) and operated stifles (IAP + and IAP-). Columns with different letters within each graph are significantly different ($P < .05$). Values are expressed as mean $\pm$ SEM. C2C, collagen II neoepitope.

Fig 7. Light photomicrographs of IAP– (A) and IAP + (B) canine cranial cruciate ligament (CrCL) midsubstance sections 12 weeks after elongation injury. All elongated CrCLs had evidence of active repair characterized by granulation tissue (G) and numerous plump fibroblasts (arrows), but granulation tissue was reduced and more organized in protected CrCL sections and collagen fibers were better aligned. (H&E, $\times 400$).

Table 1Normalized In Vivo Canine Tibial Translation ($[\text{Abs. Translation/Tibial Width}] \times 100$)

Weeks Post-op	Total Tibial Translation	Cranial Tibial Translation	Caudal Tibial Translation
IAP–			
Pre-op	5.5 ± 1.1	3.4 ± 0.86	3.3 ± 0.32
4	6.8 ± 1.3	3.6 ± 0.53	3.2 ± 1.2
8	5.9 ± 0.54	3.5 ± 0.67	3.9 ± 0.77
12	6.4 ± 1.2	2.9 ± 0.84	2.9 ± 0.84
IAP–C			
Pre-op	4.8 ± 0.83	4.2 ± 0.68	2.4 ± 0.52
4	7.8 ± 1.1	4.0 ± 0.22	3.9 ± 0.35
8	6.5 ± 0.92	3.1 ± 0.72	3.9 ± 0.70
12	4.8 ± 0.39	2.6 ± 0.66	2.7 ± 0.33
IAP +			
Pre-op	7.2 ± 0.98	3.3 ± 0.71	4.0 ± 0.39
4	6.9 ± 1.2	3.9 ± 0.64	3.7 ± 0.78
8	6.6 ± 0.53	2.8 ± 0.23	4.1 ± 0.53
12	6.7 ± 1.5	3.7 ± 0.81	3.3 ± 0.29
IAP +C			
Pre-op	7.8 ± 1.2	3.7 ± 0.69	3.9 ± 0.94
4	7.0 ± 0.79	3.1 ± 0.50	4.7 ± 0.37
8	7.0 ± 0.61	4.0 ± 0.34	3.5 ± 0.41
12	8.8 ± 1.4	5.6 ± 2.4	3.3 ± 0.60

Values (mean ± SEM) are reported for stifles with (IAP +) and without (IAP–) intra-articular protection after CrCL elongation with respective unoperated controls (IAP–C and IAP +C) preoperatively (preop) and 4, 8, and 12 weeks postoperatively (postop).

IAP, intra-articular protection; CrCL, canine cranial cruciate ligament.

Table 2

In Situ Stifle Rotation and Translation Normalized to Tibial Width with and Without Joint Capsule (JC)

	Rotation (°/mm)			Translation		
	Total	Medial	Lateral	Total	Cranial	Caudal
IAP–						
JC	33.0 ± 3.2	17.8 ± 2.6	15.2 ± 1.6	7.1 ± 2.7	3.8 ± 0.89	3.5 ± 1.9
No JC	40.7 ± 0.83	21.4 ± 2.2	19.3 ± 3.1	7.6 ± 2.6	3.5 ± 1.9	3.5 ± 2.0
IAP–C						
JC	40.9 ± 5.0	18.0 ± 1.3	22.4 ± 3.5	4.8 ± 1.1	4.7 ± 0.98	3.5 ± 0.74
No JC	44.9 ± 4.2	24.8 ± 5.8	21.6 ± 3.5	5.3 ± 0.77	3.9 ± 0.81	2.9 ± 0.33
IAP +						
JC	32.9 ± 2.9	18.8 ± 1.4	14.1 ± 2.1	8.0 ± 1.3	4.0 ± 0.48	4.0 ± 1.1
No JC	35.3 ± 3.0	20.8 ± 2.2	14.5 ± 2.4	8.7 ± 1.3	5.3 ± 0.80	3.4 ± 0.76
IAP +C						
JC	38.6 ± 4.0	21.0 ± 2.0	17.5 ± 3.2	7.5 ± 1.0	3.6 ± 0.47	3.8 ± 0.62
No JC	40.5 ± 4.5	23.3 ± 2.7	17.2 ± 2.9	8.3 ± 0.97	3.5 ± 0.27	3.5 ± 0.27

Values (mean ± SEM) are reported for stifles with (IAP +) and without (IAP–) intra-articular protection after CrCL elongation with respective unoperated controls (IAP–C and IAP +C) 12 weeks after surgery.

IAP, intra-articular protection; CrCL, canine cranial cruciate ligament.