

# In Vitro Biomechanical Testing of Equine Metacarpophalangeal Collateral Ligaments and Collateral Ligaments Repair Using Suture Anchors.

Remigiusz Maciej Grzeskowiak (✉ [remik.grzeskowiak@gmail.com](mailto:remik.grzeskowiak@gmail.com))

University of Tennessee Knoxville College of Veterinary Medicine <https://orcid.org/0000-0002-8656-5162>

Rebecca E. Rifkin

University of Tennessee Knoxville College of Veterinary Medicine

Pierre Yves Mulon

University of Tennessee Knoxville College of Veterinary Medicine

David P. Harper

University of Tennessee Knoxville Center of Renewable Carbon

H. Steve Adair

University of Tennessee Knoxville College of Veterinary Medicine

David E. Anderson

University of Tennessee Knoxville College of Veterinary Medicine

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## Research article

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# Abstract

**Background** The disruption of equine metacarpophalangeal (MCPCL) and metatarsophalangeal collateral ligament (MTPCL) occurs as a result of traumatic events and it has been associated with guarded to poor prognosis for the return to athletic performance before the injury. Rupture of the MCPCL induces joint laxity which can, directly and indirectly, lead to a cartilage damage of the metacarpophalangeal joint (MCPJ). The literature describes several methods of the surgical management of those injuries, including the suture anchors. However, the knowledge about their biomechanical performance in the equine MCPCL repair model is lacking. This study describes the method of suture anchor placement within the metacarpal bone as well as reveals data of the biomechanical testing. The first objective was to perform bending tests on equine metacarpophalangeal joints with intact metacarpophalangeal collateral ligaments. The second objective was to perform similar tests on the metacarpophalangeal joints, having completely severed metacarpophalangeal collateral ligaments which were reattached to the suture anchors. **Results** The results of the biomechanical testing of 50 specimens showed that the suture anchor repairs obtained 15% of the intact metacarpophalangeal collateral ligaments' maximum load, 34% of their bending stiffness, 14% of their load at maximum flexure extension, and 82% of the maximal flexure extension. The suture anchor placement within the bone can be performed quick and in the feasible way. The results of the biomechanical testing did not vary significantly between the tested types of the suture anchors used in this study. **Conclusions** The repairs with the suture anchors achieved lower biomechanical testing results compared with the intact metacarpophalangeal collateral ligaments; however, the anatomical position of the joint could be easily restored with the repair. The use of anchors may anatomically restore the ligament and provide additional mechanical support for the injured metacarpophalangeal collateral ligaments.

## Background

The disruption of the equine metacarpophalangeal (MCPCL) and metatarsophalangeal collateral ligament (MTPCL) most often occurs as a result of traumatic events<sup>1-3</sup>. A recent study on their anatomical and histological appearance reported that almost 61% of 319 MCPCL collected from horses without any history of orthopedic problems presented pathologic changes on histological examination including mild changes in the cellular density and collagen orientation as well as severe fibrocartilaginous metaplasia<sup>2</sup>. Another retrospective study reported 17 clinical cases with the MCPCL and MTPCL over 6 years of time period<sup>3</sup>. The equine MCPCL and MTPCL consist of a superficial (long) layer and a deep (short) layer<sup>2,3</sup>. The superficial layer extends vertically from the distal metaphysis of the third metacarpal bone (MC3) or third metatarsal bone (MT3), and the deep layer runs obliquely in the distopalmar or distoplantar direction from its origin in the epicondylar fossa of the MC3 or MT3<sup>2,3</sup>. Both layers have their distal attachment on the proximal phalanx (PP)<sup>2,3</sup>. The rupture of both components (long and short) occurs more often than the rupture of only one of them<sup>4</sup>. The uniaxial injuries occur with the similar frequency as the biaxial injuries and the fore limbs are equally affected as the hind limbs<sup>3,4</sup>.

The mechanism of injury has been attributed to a trauma, such as stepping into a hole, entrapment of a limb in a gate or fence, or injury during a high-speed event<sup>4,5</sup>.

The most commonly used method of treatment in horses utilizes limb immobilization within a distal limb fiberglass cast<sup>2</sup>. Average cast immobilization ranges from 14 to 132 days (mean 71 days)<sup>2</sup>. Limb immobilization is necessary because horses are prone to additional injuries, which contribute to the worsening of their condition. Rupture of the MCPCL induces joint laxity, loss of joint congruency, and abnormal cartilage weight-bearing forces, which can, directly and indirectly, lead to a cartilage damage of the metacarpophalangeal joint (MCPJ)<sup>6</sup>. The ongoing arthritis can be exaggerated with long-term limb immobilization<sup>7</sup>. The literature provides reports of successful attempts of MCPCL stabilization shortly after the injury, including the use of a polypropylene mesh, carbon fiber implant, or the suture anchors shortening of time spent in the cast<sup>8-10</sup>. According to the authors, the stabilization resulted in a shortening of post-surgical limb immobilization<sup>8-10</sup>. The prognosis for current MCPCL rupture treatment has been reported to be good for breeding purposes and guarded to poor for athletic performance<sup>2,5</sup>.

Commercially available suture anchors have been well described and used in human medicine. They differ in size, shape, and suture type threaded through the eyelet. This makes them useful in a variety of anatomical regions, such as the knee, foot, hand, and ankle<sup>11-15</sup>. They are, however, most often used to repair human glenohumeral instability, superior labral tear from anterior to posterior, as well as rotator cuff injuries<sup>11,12</sup>. Several studies have described their application in small animal medicine. They have been used in the repair of MCPCL insufficiencies, as well as tear of the medial glenohumeral ligament, in the fixation of the lateral suture technique in cranial cruciate ligament repair, in the reduction of coxofemoral luxation, and in the reattachment of the tendons<sup>16</sup>. Even though they are used in humans and small animals, studies about their application in horses are lacking. A literature review indicates that horses may benefit from their use in MCPCL and MTPCL injuries<sup>2, 5-10</sup>. An additional stabilization of equine ligament with the suture anchors may enhance the healing and shorten the time of limb immobilization, thus reducing the development of cartilage damage. To our knowledge, there has not been an *in vitro* MCPCL injury model in horses that investigates their biomechanical behavior, which would also serve as the model for their repair with suture anchors.

We hypothesized that ligament repair using suture anchors would partially restore the mechanical integrity of the ligament. The objective of this study was to perform biomechanical testing of intact MCPCL under 4-point bending conditions in order to determine the failure mode in our testing construct. Using these methods, the second objective was to perform the same biomechanical testing on the MCPCL repair performed with suture anchors<sup>a,b</sup> to determine the degree of stabilization provided soon after the injury and to compare their resistance to bending to intact MCPCL.

## Results

### Test specimen characteristics

The measurement of the prepared MC3 thickness at the level of the MCPCL attachment equaled 17.02 mm ( $\pm 1.10$  mm), and the prepared PP at the level of the MCPCL attachment equaled 16.11 mm ( $\pm 1.32$  mm). The mean lateral and medial MCPCL thickness were 4.75 mm ( $\pm 0.66$  mm) and 4.63 mm ( $\pm 0.56$  mm), respectively. The mean surface area of the lateral and medial MCPCL was 352.97 mm<sup>2</sup> ( $\pm 37.6$  mm<sup>2</sup>) and 357.36 mm<sup>2</sup> ( $\pm 25.67$  mm<sup>2</sup>), respectively. The mean total length of the PP was 90.11 mm ( $\pm 4.62$  mm) (Table 1). The Kolmogorov-Smirnov normality test revealed that the data was normally distributed ( $p > 0.05$ ). The independent student T-test did not reveal significant differences in MCPCL thickness and area, MC3 thickness, PP thickness, MCPCL surface area between lateral and medial specimens ( $p > 0.05$ ) (Table 2).

### **Collateral ligaments biomechanical testing** (Graph 1)

In all specimens, the observed method of failure was the fracture of the PP at the distal attachment of the MCPCL. This occurred at an average maximum load for the lateral and medial specimens,  $9.13 \pm 2.73$  kN and  $7.95 \pm 1.42$  kN, respectively (Tables 1 and 2). At maximum load, a significant disruption of the collagen fibers occurred in the center of the MCPCL. This was noticed as a translucent area in the MCPCL (Figure 1). The bending stiffness of the lateral and medial MCPCL equaled  $3.43 \pm 1.57$  kN/mm and  $4.55 \pm 2.75$  kN/mm, respectively. The load at maximum flexure extension of the lateral and medial specimens equaled  $5.12 \pm 1.52$  kN and  $4.31 \pm 0.49$  kN, respectively, and the maximum flexure extension for the lateral and medial specimens equaled  $9.12 \pm 2.05$  mm and  $8.85 \pm 1.73$  mm, respectively (Table 1). The mean thickness of the MCPCL measured along its midpoint within the MCPJ was significantly thinner after the test ( $2.63$  mm  $\pm 0.44$  mm, lateral;  $2.38$  mm  $\pm 0.43$  mm, medial) with reduction in the thickness by 44.4% and 49.6% of the lateral and medial ligament, respectively (Table 2). The translucent area of the MCPCL equaled  $133.43$  mm<sup>2</sup>  $\pm 35.22$  mm<sup>2</sup> in the lateral specimen and  $164.28$  mm<sup>2</sup>  $\pm 62.87$  mm<sup>2</sup> in the medial specimen (Table 2). Further, the translucent area in the lateral ligament was 38% of the initial surface area, and the medial ligament was 46% of the initial medial ligament surface. The PP failed in the shear-free zone between the loading points of the actuator at the distal attachment of the MCPCL. The mean length of the segment proximal to the fracture line was  $22.58$  mm  $\pm 6.16$  mm, and the distal segment was  $67.95$  mm  $\pm 7.24$  mm (Table 1). The average results of intact MCPCL mechanical testing for both medial and lateral specimens equaled  $8.54 \pm 2.19$  kN in the maximum load,  $4.72 \pm 1.17$  kN in load at maximum flexure extension, and  $8.99 \pm 1.85$  mm in maximum flexure extension. The Kolmogorov-Smirnov normality test revealed that the data was normally distributed ( $p > 0.05$ ). The Lavené's test for equality of variance did find variances within the variables to be homogenous. The two-tailed independent student t-test did not reveal significant differences in the variables between the lateral and medial side of the specimen ( $p > 0.05$ ) (Table 2). The two-tailed Pearson correlation test revealed a significant ( $p < 0.05$ ) inverse correlation between the MCPCL thickness and the PP total length ( $-0.595$ ), between the bending stiffness and the length of distal fractured fragment of the PP ( $-0.527$ ), and a positive correlation between the translucent area of the MCPCL after the test with bending stiffness ( $0.697$ ) and maximum load ( $0.526$ ) (Table 3).

### **Suture anchors biomechanical testing** (Graph 2)

The suture anchors obtained an average maximum load of 1.26 kN, which was approximately 15% of the maximum load in the intact ligament group. The average bending stiffness in the anchor group was 1.35 kN/mm (34% of the intact ligament), load at maximum flexure load was 0.66 kN (14% of the intact ligament), and the maximum flexure extension was 7.41 mm (82% of the intact ligament) (Table 4; Graph 2). The independent T-test between combined results of suture anchor testing and intact MCPCL testing revealed significant differences between both groups ( $p < 0.05$ ) (Table 4). The detailed analysis of each suture anchor type revealed that the CSW<sup>d</sup> anchor failed at highest mean maximum load ( $1.69 \pm 0.9$  kN) with below average mean bending stiffness ( $0.86 \pm 0.6$  kN/mm) (Table 5). The CSW B<sup>e</sup> failed at lower maximum load ( $1.49 \pm 0.5$  kN), with highest mean bending stiffness ( $1.18 \pm 1.0$  kN/mm) in the single suture anchors group (Table 5). The CSW II<sup>c</sup> and single IMX<sup>f</sup> failed at lowest maximum loads ( $1.07 \pm 0.6$  kN and  $0.82 \pm 0.2$  kN, respectively), and bending stiffness was calculated only for the CSW II<sup>c</sup> anchor ( $0.17 \pm 0.5$  kN/mm) (Table 5). The double IMX<sup>f</sup> failed at average maximum load ( $1.33 \pm 0.7$  kN) with highest bending stiffness (1.40 kN/mm) (Table 5). The Kolmogorov-Smirnov normality test revealed that the data of the mechanical testing of suture anchors was not normally distributed ( $p < 0.05$ ). Non-parametric Kruskal-Wallis test did not reveal any significant differences in maximum load, bending stiffness, load at maximum flexure extension, as well as maximum flexure extension between the different anchor groups ( $p > 0.05$ ). The non-parametric Mann-Whitney U and Wilcoxon W test did not reveal significant differences in the results of the mechanical testing between the single and double IMX<sup>f</sup> suture anchor ( $p > 0.05$ ). The most common modes of failure included eyelet disruption (15/32), suture tear (14/32), and ligament tear (3/32). The Fisher's exact test revealed a significant correlation between the type of the suture anchor and the method of failure ( $p < 0.05$ ). The eyelet disruption was significantly correlated with the CSW II<sup>c</sup> and CSW<sup>d</sup> suture anchors, whereas the suture break was significantly correlated with the IMX<sup>f</sup> suture anchors and ligament tears with the CSW B<sup>e</sup> suture anchors.

## Discussion

This is the first study to investigate the bending stiffness and flexure strength of equine MCPCL. The biomechanical testing of MCPJ with intact MCPCL resulted in a PP bone failure prior to the ligament failure. The bone fracture occurred at the distal attachment of MCPCL in the PP. The fractures were found in the zone between the loading points, which is considered as the shear free zone. The four-point bending test causes not only the strain and tensile stress on the convex side of the specimen, but also the compressive stress and strain on the concave side of the specimen<sup>17</sup>. This stress causes PP fracture as a result of the combined stress which occurred in the specimen during the failure. The testing rate (1 mm/sec) is a standard loading rate for acute load to failure testing in our laboratory, and it was set up carefully to reduce the combined stress on MC3 and PP bone and, therefore, allow for the ligament testing. The same ligament testing rate was previously reported in the *in vitro* model of the UCL injury in humans<sup>15</sup>. The length of PP fragment distal to the fracture was negatively correlated with the bending stiffness of the specimen. The fracture line tended to be more proximal and closer to the distal attachment of MCPCL in the more compliant specimens (lower bending stiffness) and more remote to it

in the less compliant specimens. Further, a significant negative correlation was found between the length of the PP and initial thickness of the MCPCL. This suggests that horses with a longer pastern in our study had thinner MCPCL. This may be determined during a following investigation assessing the risk of MCPCL rupture. Besides the PP fracture, the biomechanical testing resulted also in the disruption of the collagen fibers, which was apparent as the translucent area within the MCPCL.

The four-point bending test has been widely used in biomechanical testing<sup>19-22</sup>. The advantage of 4-point bending is related to the equal distribution of the maximum bending moment between the loading points during the loading<sup>17</sup>. The MCPCL, as well as suture anchor, was placed in the center between the loading points, which were equal distance from the proximal and distal attachment of the MCPCL. This allowed equal distribution of the maximum bending point, thus the bending stress along the entire length of the MCPCL during the testing. The three-point bending test as opposed to the 4-point bending test would have concentrated the bending stress at the point of application load, which would have been in the center of MCPCL, and it would have skewed the results of the biomechanical testing<sup>17,22</sup>. The bending test was chosen over a simple tension test because this study aimed to test the MCPCLs under *in vitro* conditions, which were as close to the *in vivo* conditions as possible. Under *in vivo* conditions, the acute MCPCL injury results not just because of the simple tensile stress but because of the combined bending stress caused in the MCPJ<sup>3-5</sup>.

There was no statistical difference ( $p > 0.05$ ) in biomechanical characteristics between the lateral and medial specimens containing the intact MCPCLs. However, the testing of the lateral specimens resulted in a greater mean maximum load and lesser mean bending stiffness as compared with the medial specimens. In other words, the lateral specimens were more compliant and stronger when compared with the medial side. Interestingly, the greater maximum load was associated with a smaller area of collagen fiber disruption in the lateral MCPCL (133.43 mm<sup>2</sup>) as compared with the medial MCPCL (164.28 mm<sup>2</sup>). Several studies have reported that the lateral MCPCL tends to be injured more frequently than the medial MCPCL<sup>1-3</sup>. Pohlin et al. (2014) found significantly more pathologic changes (from mild changes in the cellular density and collagen orientation to severe fibrocartilaginous metaplasia) in the lateral MCPCL compared with the medial MCPCL on histologic examination<sup>2</sup>. The changes were explained as adaptive responses to stress during normal locomotion<sup>2</sup>. In contrast, another study did not find differences between measured stress in the lateral and medial sides of the MCPJ under a variety of loading scenarios and pressure distributions (from 1.8 kN to 12 kN)<sup>23</sup>. The intraarticular pressure changes measured on the lateral and medial side under distinct loads (pressure changed from 0.01 - 0.015 kN/mm to 0.03 - 0.035 kN/mm) also did not differ between the sides<sup>23-24</sup>. It is important to note that those studies were performed under static *ex vivo* conditions. This suggests that the adaptive changes in the ligaments occur as a response to cyclic events more so than a single event. The complex stabilization structures of the MCPJ (sagittal ridge, proximal sesamoid bones ligaments, joint capsule, and suspensory ligament) allow for not only sagittal motion of the joint, but also for some degree of a rotation during physiologic locomotion. Therefore, the loading conditions under *ex vivo* conditions are hard to mimic in the laboratory<sup>24</sup>.

The detailed analysis of the suture anchors biomechanical performance revealed that all obtained similar results. The CSW<sup>d</sup> failed at higher mean maximum load with below average bending stiffness as compared with the remaining suture anchors; however, the statistical analysis did not reveal significant differences ( $p > 0.05$ ) between the anchor types or the number of anchors placed in the bone. The calculated differences were not significant due to the large variance within the groups. This variance resulted most probably from the anisotropic properties of the bone as well as the ligament. Furthermore, this variance could be related to the fact that the anchors were placed by two different investigators, and this could have impacted the way how the anchors were placed in the bone.

The results of suture anchors biomechanical performance were further combined and compared with the results of intact MCPCL biomechanical performance, since there was no statistical difference between the types of the suture anchor. The average values of combined suture anchors results were significantly different from the intact MCPCL and obtained 15% of the intact MCPCL's maximum load, 34% of their bending stiffness, 14% of their load at maximum flexure extension, and 82% of their maximum flexure extension. In other words, the suture anchors failed at a lower bending load as compared with the intact MCPCL, had lower flexure strength, and had lower resistance to bending. The suture anchors, however, achieved nearly the same maximum flexure extension as the intact ligaments before failing. This means that they failed at the same displacement of the actuator during the test, which supports the method of repair used to restore the physiologic relationship of the MC3 and the PP. The lower flexural strength of the suture anchors resulted from the lower fiber stress, which they can tolerate before failure. This is related to a significantly lower circumference of the fiber's area as compared with the intact MCPCL, which are significantly thicker. Nevertheless, the suture anchor's failure to load in this study is comparable to other biomechanical studies on the ulnar carpal ligament (UCL) repair in humans. The evaluated two suture anchor repairs of the UCL obtained 22% of the maximum load of intact UCL (0.379 kN)<sup>25</sup>.

Under *in vivo* condition, the weakest point in the soft tissue reattachment to the anchor is the suture or failure of the suture-soft tissue interface<sup>26</sup>. In our study, three different methods of mechanical failure of repair were observed: eyelet disruption, suture disruption, and ligament tear. Statistical analysis found a significant correlation between the anchor type and certain methods of failure. These methods of failure resulted from the specific design of each anchor type, which varied between the types. The methods of failure are consistent with the other *in vitro* studies in which they were defined in the same manner<sup>11,27</sup>.

The method of anchor placement within the bone is important. According to the literature, the suture anchors have higher pullout strength when they are placed further away from the joint surface (3 to 5 cm compared with 1 cm) due to the thicker cortical bone<sup>27</sup>. On the other hand, a better stabilization of the reattached ligament is achieved when the suture anchor is placed at the site of ligament attachment<sup>10</sup>. The anchors in our study were placed in the epicondylar fossa, approximately 2.5 cm away from the joint surface, and none of them pulled out from the bone. The anchors were also placed at a 35-45 degree angle towards the MCPCL to minimize the friction of the suture against the anchors' surface and, therefore, reduce the risk of the suture tearing. This was achieved for all the anchors except for the IMX

anchors with the eyelet made from stainless steel, which is known to reduce the suture's resistance to friction. It is also important to note that it is unclear how actuator loads relate to physiological ligament loads.

The MCPCL were reattached to the epicondylar fossa using two locking-loop suture patterns. This method was relatively easy and readily performed by the investigators, and it has been previously described<sup>10</sup>. Comparison of different suture patterns for tendon repairs found that the locking-loop pattern was mechanically superior to the Mason-Allen and Krackow patterns, and it does not result in dramatic tendon constriction nor compromise to the tendon vascularity<sup>28,29</sup>. In vitro, the locking-loop pattern is mechanically inferior to the 3-loop pulley<sup>30</sup>, but the 3-loop pulley is technically difficult to reattach the MCPCL to the epicondylar fossa. In the current study, only three specimens failed through the ligaments. The study concerning rotator cuff repair concluded that the suture pattern used to repair the ligament is not as important as the number of sutures threaded through the ligaments, and the number of sutures is positively correlated with the strength of the new repair<sup>31</sup>.

The limitations of the current study are mostly related to the anisotropic properties of the specimens used in our study (bone and ligament), and the geometry of tested specimens were not constant and easily defined between specimens. Additionally, the bending apparatus used was designed for materials of uniform cross-section. Further study is necessary to calculate material properties of the MCPCL from this testing configuration for joints of highly variable geometry. Furthermore, the study is limited by the relatively low number of suture anchors available for testing. This was addressed with the non-parametric statistical test. The focus of this investigation, however, was to establish an *in vitro* model of MCPCL injury and test how the MCPCL repair performed with the four most commonly used types of the suture anchors. Finally, in vitro conditions cannot be directly translated to the in vivo conditions; however, this study provides useful data regarding use of suture anchors in repair of MCPCL injuries to guide future *ex vivo* and *in vivo* studies.

## Conclusions

This model of *in vitro* MCPCL injury resulted in the MCPCL rupture and PP failure. The suture anchors used to repair the injury partially supported MCPCL repair and may support use of such implants to provide additional support for MCPCL injuries. The MCPJ is a complex articulation, which involves multiple structures responsible for joint stability (sagittal ridge, proximal sesamoid bones ligaments, joint capsule, and suspensory ligament). These structures will contribute to fetlock stability during healing of the MCPCL. The fetlock articulation allows for shielding of the MCPCL from the main forces of weight bearing that are applied to the proximal phalangeal joint surface during physiologic locomotion. Further biomechanical tests are required to evaluate this surgical method, which would result in the soon post-surgical stabilization of the joint. Horses may benefit from this procedure by reducing time required for immobilization and, thus, reducing the severity of osteoarthritis that occurs following prolonged immobilization.

# Materials And Methods

## Specimen preparation

Twenty-five equine forelimbs were included in the study. The limbs were harvested from adult horses (ages ranging from 6 to 14 years and weight ranging from 500 to 700 kg), which were euthanized for reasons unrelated to this study. The forelimbs were harvested at the level of the mid-metacarpus and proximal interphalangeal joint (PIPJ) shortly after euthanasia. All soft tissues were removed, leaving the MCPCL intact. The bones were then divided in the sagittal plane, making 50 specimens in total. Each medial and lateral MCPCL was randomly assigned to the treatment group, and it was tested separately. The triangle bone wedges containing the sagittal ridges were removed from the MC3, and similarly, the corresponding triangle bone wedges were removed from the proximal phalanx (PP; Figure 2). This was performed to minimize the bone contact and eliminate the compression and strain of the sagittal ridge during the test, so the bending of the specimen resulted in the maximum tensile stresses and stress applied to the MCPCL. During the specimen preparation, care was taken that the contact area of MC3 articular cartilage surface and adjacent PP cartilage were equal (Figure 2). Each specimen was labeled as lateral or medial, containing the respective MCPCL. The thickness of MC3 and PP were measured in three different spots along the cut edges of the bones at the proximal and distal attachment of the MCPCL, while the digital caliper and the average thickness was recorded. The MCPCL thickness was measured at the midpoint along its length within the MCPJ space. In 18 intact specimens, biomechanical testing was used to determine the characteristics of their failure mode. The remaining 32 specimens were randomly assigned to one of 6 suture anchors (CSW II<sup>c</sup>, CSW<sup>d</sup>, CSW B<sup>e</sup>, IMX<sup>f</sup> single, as well as double). The anchors used for the study included 5.5 mm CSW II<sup>c</sup> with USP No. 2 braided polyethylene suture<sup>g</sup> (n = 8), 5.5 mm CSW<sup>d</sup> with USP No. 2 braided polyethylene suture<sup>g</sup> (n = 8), 5.5 mm CSW B<sup>e</sup> with USP No. 2 braided polyethylene suture<sup>g</sup> (n = 4), and IMX<sup>f</sup> with USP No. 6 monofilament polyamide suture<sup>h</sup> single anchor (n = 7) and double anchor (n = 5).

## Collateral ligaments repair using suture anchors

The specimens assigned to the anchor test groups were further dissected, separating the MCPCL from its proximal attachment to the epicondylar fossa on the MC3. The anchors were placed in the epicondylar fossa in a 35-45 degree angle towards the ligament to reduce the suture friction against the anchor's surface. The anchors were placed in the bone according to the user's manual. The bone was drilled using a 3.5 mm drill bit<sup>i</sup> under a 35-45 degree angle to the axial bone surface. The drilled hole for CSW<sup>c-e</sup> suture anchors only was subsequently tapped with the tapping device<sup>j</sup>. Each anchor contained four USP No. 2 braided polyethylene sutures<sup>g</sup>. The IMX<sup>f</sup> suture anchors were also placed accordingly to user's manual. The bone was drilled with a 3.5 mm drill bit under a 35 to 45 degree angle and tapped with the tap device<sup>k</sup>. 4.5 mm IMX<sup>f</sup> suture anchors were placed in the bone, and a USP No. 6 pseudo-monofilament polyamide suture<sup>h</sup> was threaded through the eyelet. The ligaments were reattached using the 2 locking loops suture pattern (Figure 4). The sutures were threaded separately through the ligament using a 22-

gauge needle. Sutures were tied pairwise with 7 throws (Figure 4). Care was taken to ensure that the loops were separated as remotely from each other as possible, and the proximal loop was narrower than the distal loop (Figure 3). The MCPCL were reattached to the suture anchor, maintaining the physiological position and relationship of the MCPJ and the MC3 and PP (Figure 4).

### **Collateral ligaments biomechanical testing**

Specimens were analyzed utilizing a 4-point bending test apparatus on an electromechanical universal testing machine<sup>l</sup> coupled with a 30 kN maximum actuator. The test allowed placement of the MCPCL under maximum strain and tensile stress, distributing the maximum bending moment equally along the length of the MCPCL between the loading points. Specimens were fixed on the machine between the supports for the 4-point bending test, with the MC3 on the left and PP on the right side and, therefore, with the MCPCL directed to the bottom of the machine (Figure 2). Care was taken to position the MCPCL directly in the middle under the actuator, with the loading points positioned symmetrically and equally distanced from the proximal and distal MCPCL attachment. Furthermore, the loading points were separated from the supports as much as possible to minimize the stress caused on the specimens between them. The tests were performed with the loading rate of 1mm/sec until the specimen failed. The mode of failure was recorded as a bone fracture and/or a collagen fiber disruption, which was characterized as the translucent area within the MCPCL and loss of the MCPCL thickness. Eighteen intact MCPCL specimens underwent the mechanical tests, including nine lateral MCPCL and nine medial MCPCL. The testing parameters involved the maximum load, which was expressed in kilonewtons (kN), and it was defined as the maximum load applied by the actuator to the specimen during the test right before specimen's failure. The displacement of the actuator was measured as a relative distance (mm) of the actuator to its' original location at the start of the test. The irregular geometry and nonuniformity from specimen to specimen prevented an accurate measurement of the modulus in this study. The bending stiffness of the specimens was measured, and this characterized specimens' resistance to bending under the load applied by the frame during its continuous displacement throughout the test. The bending stiffness was expressed in kilonewtons over millimeters (kN/mm). The flexural strength of tested specimens was defined as the maximum stress developed in the specimens during loading prior to failure. The flexural strength was measured with the load applied by the actuator, which placed the MCPCLs collagen fibers or the anchored suture under the maximum fiber stress and the entire specimen under its maximum extension before the failure (load at maximum flexure extension). The last mechanical parameter recorded in this study measured the displacement of the actuator, when the specimen was placed under the maximum extension (maximum flexure extension). A video of each test was recorded, and the test results were analyzed using the manufacturer's software<sup>m</sup>.

### **Suture anchors biomechanical testing**

The MCPCL repaired with the suture anchors were placed on the holding frame in the same manner as the intact MCPCL specimens. The anchor groups consisted of 32 specimens (8 CSW II<sup>c</sup>, 8 CSW<sup>d</sup>, 4 CSW B<sup>e</sup>, 7 IMX<sup>f</sup> single, and 5 IMX<sup>f</sup> double). The specimens were positioned so the maximum bending moment

was applied across the suture anchor. Again, care was taken while setting up the load points of the actuator to minimize applied shear force. The tests were performed with the same settings as the tests for the intact MCPCL, and identical parameters were recorded. The construct failure mode was noted as an eyelet disruption, a suture disruption, and a suture pullout through the ligament causing a ligament tear.

## **Statistical analysis**

The analysis was performed using statistical software<sup>n</sup> and power and specimen calculation software<sup>o</sup>. Descriptive statistics were determined for all variables in the intact ligament testing group, including minimum variable value, maximum variable value, mean, standard deviation, variance, and skewness (statistic, standard error of skewness). The normality of data distribution in each variable was performed using a Kolmogorov-Smirnov test of normality. The lateral and medial specimens test results were compared using the two-tailed independent samples T-test. The correlations between all variables were calculated using the two-tailed Pearson correlation test.

Descriptive statistics were calculated for all variables in the anchor testing group, including minimum variable value, maximum variable value, mean, standard deviation, variance, and skewness (statistic, standard error of skewness). The normality of data distribution in each variable was performed using Kolmogorov-Smirnov test of normality. Because they are not normally distributed data in the anchor testing, the results of their performance, including maximum load (kN), bending stiffness (kN/mm), load at maximum flexure extension (kN) and maximum flexure extension (mm), were compared to the results of the MCPCL biomechanical test using the two independent samples T-test. The differences in the mean values of the testing results between the anchor groups were calculated using a non-parametric Kruskal-Wallis test and between single and double suture anchor using the non-parametric Mann-Whitney U, as well as Wilcoxon W test. The method of failure was correlated with the anchor type using a Fisher's-Exact test. The level of significance was established at the  $p < 0.05$  and the power of the study as  $\beta = 0.9$ .

## **List Of Abbreviations**

kN – kilonewton

MC3 – third metacarpal bone

MCPCL – metacarpophalangeal collateral ligaments

MCPJ – metacarpophalangeal joint

kN/mm – kilonewton per millimeter

MT3 – third metatarsal bone

MTPCL – metatarsophalangeal collateral ligaments

mm/min – millimeter per minute

mm/sec – millimeter per second

PIPJ – proximal interphalangeal joint

PP – proximal phalanx

UCL – ulnar collateral ligament

USP – United States Pharmacopeia

## **Declarations**

### **Ethics approval and consent to participate**

Not Applicable

### **Consent for publication**

Not Applicable

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing Interest**

The authors declare no conflicts of interest.

### **Founding**

Not Applicable

### **Author Contributions**

RMG organized and analyzed data, prepared the specimens, performed the suture anchor placement and the biomechanical testing as well as wrote the manuscript. RER assisted with the specimen preparations and suture anchor placement. PYM and DPH created the methods of the biomechanical testing and performed the testing as well as assisted with data analysis. HSA and DEA mentored the project as well as supervised the data collection, analysis and manuscript writing.

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## Endnotes

1. Arthrex®, Inc. 1370 Creekside Blvd. Naples, Florida 34108 USA
2. IMEX® Veterinary, Inc. 1001 McKesson Drive, Longview, Texas 75604 USA
3. Arthrex® Corkscrew FT II™, Naples, Florida 34108 USA
4. Arthrex® Corkscrew FT™, Naples, Florida 34108 USA
5. Arthrex® Corkscrew FT Bio™, Naples, Florida 34108 USA
6. IMEX® Suture Anchor, Longview, Texas 75604 USA
7. Arthrex® FiberWire® suture, Naples, Florida 34108 USA
8. Braunamid® Aesculap AG, Am Aesculap-Platz, Tuttlingen 78532 Germany
9. Medical Hollow Electric Orthopedic Bone Drill, ShenZhen, China 0755-27845448
10. Punch/Tap device for 5.5 mm Corkscrew™ Arthrex ®, Naples, Florida 34108 USA
11. IMEX® Tapping device for the suture anchors, Longview, Texas 75604 USA
12. Instron® 5567 electromechanical universal testing machine, 825 University Ave, Norwood, Massachusetts 02062-2643 USA
13. Bluehill® Universal Software, Norwood, Massachusetts 02062-2643 USA
14. IBM® SPSS Statistics™ v.25 Software, 1 New Orchard Road Armonk, New York 10504-1722 USA
15. PS Power and Specimen Size Calculations ®, version 3.0, January 2009 Copyright © 1997-2009 by William D. Dupont and Walton D. Plummer

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## Tables

**Table 1.** Anatomic and biomechanical characteristics of normal metacarpophalangeal collateral ligament (MCPCL).

The descriptive statistics include mean values of both lateral and medial MCPCL as well as minimum, maximum, standard deviation and number of samples. The mean thickness of both, lateral and medial MCPCL after the testing was approximately 52% of the thickness before the testing. The bottom of the table represents the average results of both MCPCL biomechanical testing. Following abbreviations were used in the table, including third metacarpal bone (MC3), proximal phalanx (PP), and average (Avg). The results are

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
MC3 Avg Thickness (mm)	18	15.40	19.38	17.03	1.10
PP Avg Thickness (mm)	18	13.84	19.84	16.11	1.32
MCPCL Thickness (mm)	18	3.60	5.67	4.69	.59
PP Total Length (mm)	18	83.70	101.81	90.11	4.62
MCPCL Avg Thickness After the Test (mm)	18	1.86	3.35	2.51	.45
MCPCL Surface Area (mm <sup>2</sup> )	18	309.68	433.82	355.16	31.31
MCPCL Lucency Transparent Area (mm <sup>2</sup> )	18	74.52	312.06	148.86	51.92
Bending Stiffness (kN/mm <sup>2</sup> )	18	1.346	11.148	3.99	2.25
Maximum Load (kN)	18	5.97	13.69	8.54	2.19
Load at Max Flex Ext (kN)	18	3.28	7.96	4.72	1.17
Max Flex Ext (mm)	18	6.36	12.40	8.99	1.85

expressed in kilonewtons (kN), kilonewtons per millimeters (kN/mm), millimeters (mm), or square millimeters (mm<sup>2</sup>).

**Table 2.** The measurements comparison between left and right metacarpophalangeal collateral ligament (MCPCL) before and after testing.

The results of the statistical T-Test show the differences between the lateral and medial specimens. The differences in the following parameters were not statistically significant between the medial and lateral specimens. Following abbreviations were used in the table, including third metacarpal bone (MC3), proximal phalanx (PP), and average (Avg). The results are expressed in kilonewtons (kN), millimeters (mm), kilonewtons per millimeters (kN/mm) and square millimeters (mm<sup>2</sup>).

**Table 3.** The significant correlations between the measurements.

The results of the 2-tailed Pearson correlation test between the anatomical and biomechanical variables. The table shows only the significant correlations. The abbreviations used in this table include metacarpophalangeal collateral ligament (MCPCL),

2-tailed independent specimens T Test between Lateral and Medial MCPCL					
	Specimen Side	N	Mean	Std. Deviation	T Test (sig.)
MC3 Avg Thickness (mm)	Lateral	9	16.75	1.21	0.299
	Medial	9	17.30	0.98	
PP Avg Thickness (mm)	Lateral	9	15.91	0.90	0.539
	Medial	9	16.31	1.68	
MCPCL Thickness (mm)	Lateral	9	4.75	0.66	0.682
	Medial	9	4.63	0.56	
PP Total Length (mm)	Lateral	9	90.79	5.23	0.548
	Medial	9	89.43	4.11	
MCPCL Thickness After Test (mm)	Lateral	9	2.64	0.44	0.233
	Medial	9	2.38	0.43	
MCPCL Surface Area (mm <sup>2</sup> )	Lateral	9	352.97	37.60	0.776
	Medial	9	357.36	25.67	
MCPCL Lucency Area (mm <sup>2</sup> )	Lateral	9	133.43	35.22	0.217
	Medial	9	164.28	62.87	
Bending Stiffness (kN/mm)	Lateral	9	3.43	1.57	0.308
	Medial	9	4.55	2.75	
Max Load (kN)	Lateral	9	9.13	2.73	0.265
	Medial	9	7.95	1.42	
Load at Max Flex Ext (kN)	Lateral	9	5.13	1.52	0.143
	Medial	9	4.31	0.49	
Max Flex Ext (mm)	Lateral	9	9.12	2.05	0.771
	Medial	9	8.85	1.74	

proximal phalanx (PP), load at maximum flexure extension (Load at Max Flex Ext), proximal (prox), and distal (dist).

**Table 4.** The comparison of the intact metacarpophalangeal collateral ligaments (MCPCL) and suture anchor biomechanical testing.

The table shows the average results of the intact MCPCL biomechanical testing compared with the results of MCPCL attached to the suture anchors. The difference between the intact MCPCL and the MCPCL attached to the suture anchors was statistically significant in

<b>2-tailed Pearson Correlation (p &lt; 0.05)</b>		
MCPCL thickness	PP total length	r = -0.595
Ratio MCPCL lucency to MCPCL surface	PP prox length	r = -0.507
Ratio MCPCL lucency to area	MCPCL thickness after the test	r = 0.564
PP dist length	Load at Max Flex Ext	r = 0.487
PP total length	Load at Max Flex Ext	r = 0.551
MCPCL lucency area	Ratio PP prox to dist	r = 0.509
MCPCL lucency area	Ratio PP prox to total	r = 0.560
MCPCL lucency area	Bending stiffness	r = 0.697
MCPCL lucency area	Maximum Load	r = 0.526
MCPCL lucency area	Ratio PP prox to dist	r = 0.509
MCPCL lucency area	Ratio PP prox to total	r = 0.560
MCPCL lucency area	PP prox length	r = 0.497
Load at Max Flexion	PP distal length	r = 0.487
Load at Max Flexion	PP total length	r = 0.551
Load at Max Load	PP total length	r = 0.498
Bending stiffness	Ratio PP prox to dist	r = 0.486
Bending stiffness	Ratio PP prox to total	r = 0.544
Bending stiffness	Ratio MCPCL before to MCPCL after	r = 0.520
Bending stiffness	PP distal length	r = -0.527

<b>2-tailed independent specimens T Test between MCPCL and Anchor</b>						
	MCPCL or Anchor	N	Mean	Std. Deviation	Percent of intact MCPCL	T Test (sig.)
Bending Stiffness (kN/mm)	MCPCL	18	3.99	2.25	100%	0.001
	Anchor	10	1.35	4.08	34%	
Maximum Load (kN)	MCPCL	18	8.54	2.19	100%	0.000
	Anchor	32	1.26	.69	15%	
Load at Max Flex Ext (kN)	MCPCL	18	4.72	1.17	100%	0.000
	Anchor	29	.66	.41	14%	
Max Flex Ext (mm)	MCPCL	18	8.99	1.85	100%	0.039
	Anchor	32	7.41	2.82	82%	

all tested variables. The abbreviations used in this table include load at maximum flexure extension (Load at Max Flex Ext), and maximum flexure extension (Max Flex Exit). The results are expressed in kilonewtons (kN), kilonewtons per millimeters (kN/mm), and millimeters (mm).

**Table 5.** The biomechanical performance of each suture anchor type.

Anchor	Force at Maximum Load (kN)		Bending Stiffness (kN/mm)		Force at Maximum Flexure extension (kN)		Maximum Flexure Extension (mm)	
CSW II	1.07	±0.6	0.17	±0.5	0.54	±0.4	7.72	±3.0
CSW	1.69	±0.9	0.86	±0.6	0.79	±0.6	7.70	±2.9
CSW B	1.49	±0.5	1.18	±1.0	0.84	±0.5	10.30	±1.4
IMX single	0.82	±0.2			0.85	±0.1	5.57	±1.7
IMX double	1.33	±0.7	1.40		0.75	±0.4	6.75	±3.2

The table contains the average values of each biomechanical parameter. The repair performed with the CSW suture anchor failed at the highest load applied to the specimen and the repair performed with the two IMX suture anchors showed the highest bending stiffness. The difference between the repairs performed with each suture anchor type was not statistically significant. The abbreviations used in this table include the commercial names of suture anchors, Arthrex® Corkscrew FT II™ (CSW II), Arthrex® Corkscrew FT™ (CSW), Arthrex® Corkscrew FT Bio™ (CSW B), and IMEX® Suture Anchor (IMX). The results are expressed in kilonewtons (kN), kilonewtons per millimeters (kN/mm), and millimeters (mm).

## Figures



**Figure 1**

The difference in the metacarpophalangeal collateral ligament (MCPCL) appearance before the test (left image) and after the test (right image). The rupture of the collagen fibers of the MCPCL was present as the translucent area within the ligament as well reduction of the MCPCL thickness after the test.



**Figure 2**

The positioning of the specimen between the 4-point bending loading points. The third metacarpal bone (MC3) is directed to the left and the proximal phalanx (PP) is directed to the right with the metacarpophalangeal ligament (MCPCL) directed to the bottom of the machine. The MCPCL was positioned in the center with its proximal and distal attachment symmetrical distanced from the loading points. The loading points span was 70 mm and the holding points span was 120 mm.



**Figure 3**

The positioning of the locking loops during the repair. The suture anchor was placed in the epicondylar fossa of the MC3 and ligament was reattached to the suture anchor with two locking loops. A care was taken to place the locking loops as remotely to each other as possible. Proximal (MC3) is to the top and distal (PP) to the bottom of the image.



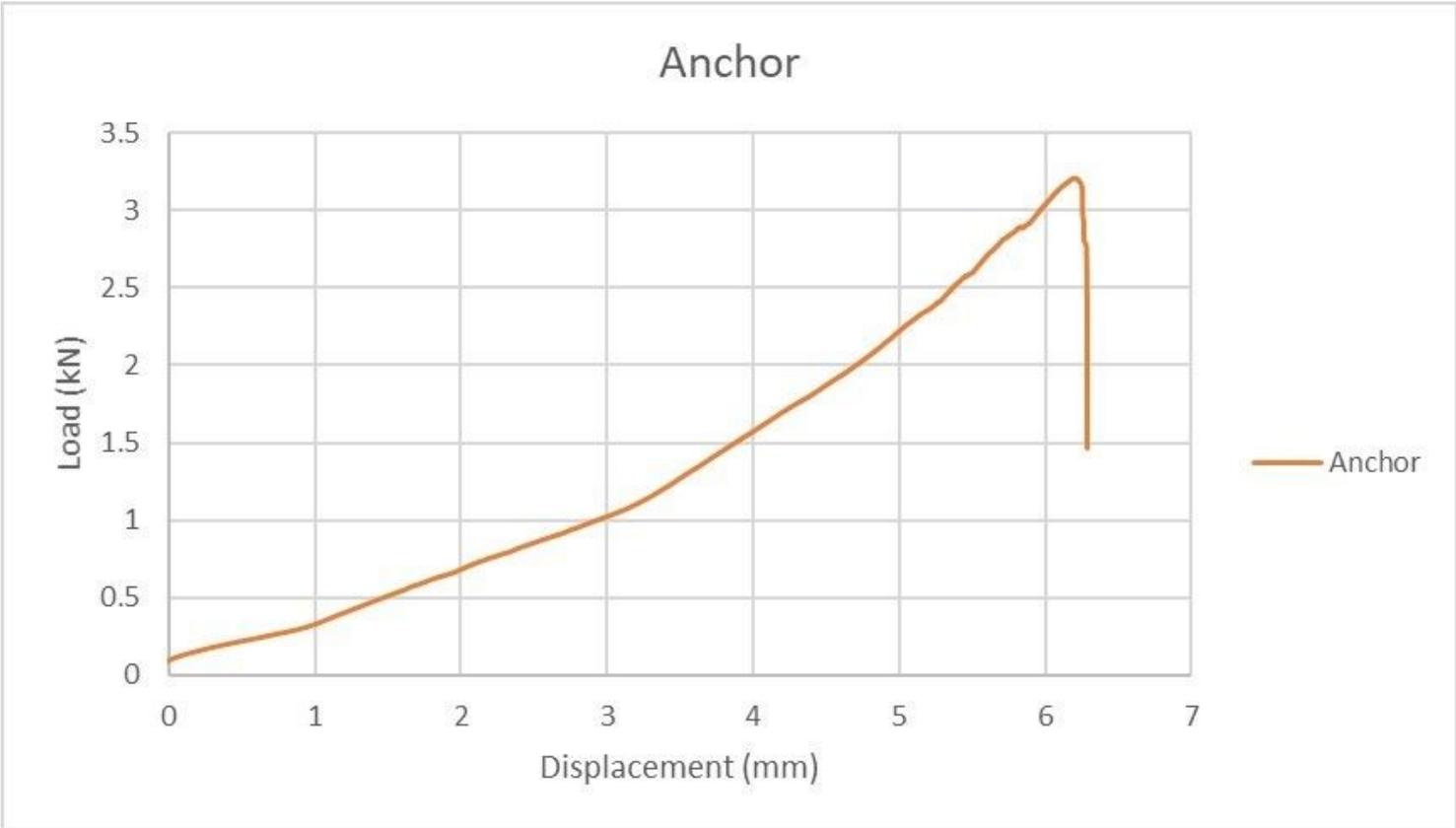
**Figure 4**

The MCPCL repair performed with the suture anchors. The left image shows the anterior-posterior projection. The repair restored the anatomic relationship between the MC3 and PP. The right image shows the latero-medial projection. The MC3 is to the top and PP to the bottom.



**Figure 5**

Graph 1: The bending stiffness of intact ligament in the 4-point bending test. The X-axis represents the actuator displacement during the test until the ultimate failure of testing sample. The Y-axis represents the load applied by the actuator to the sample during the testing. All the intact MCPCL specimens failed at the distal attachment of the MCPCL to the proximal phalanx.



**Figure 6**

Graph 2: The representative graph of bending stiffness curve of the specimen repaired with the suture anchor during the 4-point bending test. The X-axis represents the actuator displacement during the test until the ultimate failure of testing sample. The Y-axis represents the load applied by the actuator to the sample during the testing. The method of failure was associated with the type of suture anchor used to attach the MCPCL.