Preprint of: Dembski, M., Kawa, P., Tarnawski, J., Ceynowa, M., Żerdzicki, K., & Kłosowski, P. (2025). The tensile strength of different methods of anterior cruciate ligament graft end stitches in an animal model. KNEE, 53, 293-301. https://doi.org/10.1016/j.knee.2025.01.012

The tensile strength of different methods of anterior cruciate ligament graft end stitches in an animal model

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Main Text Word Count: 2461

Abstract Word Count: 246

ABSTRACT

Background: There are several methods for stitching graft ends in anterior cruciate ligament reconstructive surgery. The tendon-suture construct should be able to withstand tensioning until the graft is stabilized with an implant.

Methods: In this biomechanical study, 40 porcine lower extremity tendons ends were stitched with No. 2 suture, secured to the grips of the Zwick-Roell testing machine and tested for load at failure and type of failure (tendon v/s suture thread). The applied force was linear, the results are given in Newtons (N) as a mean. The Mann-Whitney U test was used for statistical analysis. The following constructs were compared: a whipstitch of each tendon end individually with 3, 4, and 5 passes through the tendon, a whipstitch of both tendon ends folded together and a tendon end knot without stitching.

Results: All specimens survived the minimum tensile load of 80 N. The load at failure for whipstitch with 3,4 and 5 passes were 175 N, 211 N and 254 N respectively. The load at failure was greater for individual whipstitch than for both ends folded together (261 N v/s 152 N). The mean load at failure for braided graft was 209 N. The braided graft slips off the tendon before failure leading to uneven strength distribution during tensioning.

Conclusion: Increasing number of suture passes resulted in higher load at failure. Individual tendon ends whipstitched with 5 passes was the strongest construct. All of the investigated techniques are sufficient to withstand the suggested optimal graft tension of 80 N.

Keywords: anterior cruciate ligament reconstruction, biomechanical study, animal model study

1. Introduction

Anterior cruciate ligament (ACL) tear is one of the most common sports-related injury, accounting for more than 50% of all knee injuries[1]. Although primary ligament repair is a treatment option, ACL reconstruction is currently considered the gold standard, with approximately 400,000 ACL reconstructions performed annually in the USA alone[2].

The materials used for reconstruction are either the patient's own tendons (autografts) or tendons harvested from human cadavers (allografts). Autografts, which can be either hamstring, patellar tendon or quadriceps tendon, are more commonly used[3].

Prior to anchoring the graft in the drilled femoral and tibial tunnels, it must be properly prepared, so that it can withstand tension that will be applied during implant stabilization.

Biomechanical studies have shown that an initial graft tension of 80N is recommended to prevent postoperative laxity [4–6].

Suturing the graft in a whipstitch fashion is the current gold standard of preparation, nevertheless there are other available options, some with economic, safety and time-saving advantages[7].

This study explores whether increasing the number of loops in whipstitch suture correlates with the tensile strength of the graft, if the needleless grasping technique provides adequate tensile strength, and whether suturing both ends of the grafts with a single whipstitch suture ensures sufficient tensile strength.

In this study a porcine model of tendon graft was used because their high availability, low cost and ethical considerations that are associated with human model . This model was used , some biomechanical studies have been conducted in animal models[18,19]. Similarly, our study used porcine extensor digitorum tendons, a commonly used substitute for human semitendinosus graft in ACL reconstruction research[20,21].

2. Materials and methods

2.1. Materials

Sixty fresh-frozen porcine extensor tendons were harvested from six swine's anterior legs in a typical manner, using surgical blades. The limbs were purchased from a commercial slaughterhouse fresh, frozen (- 20° C) until the day of testing. On the testing day, the legs were thawed at room temperature for 12 hours. The tendons were prepared for testing shortly after being harvested. The average age of the swine was 2 years. The study was approved by the local Bioethics Committee.

2.2 Grafts preparation

After harvesting, the tendons were cleared of all adherent tissues and covered with saline-soaked gauze to keep them moist. The tendons were then randomly divided into groups for comparison (10 specimens for each type of tendon end stitch):

1. A whipstitch of each tendon ends individually v/s a whipstitch of both tendon ends folded together.
2. A whipstitch of tendon ends with 3 v/s 4 v/s 5 passes (loops) through the tendon.
3. A tendon end knot without stitching (Braided Grafts) was compared with the results from group 2.

All suture constructs were placed 1 cm away from the end of the tendon using Novosyn No.2 suture (B. Braun SE, Melsungen, Hessen, Germany).

2.2.1 3 v/s 4 v/s 5 Loops Whipstitch Grafts

Both ends of each tendon were stitched separately using the whipstitch technique with three, four, or five loops (Fig. 1A).

2.2.2 Braided Grafts

Both ends of each tendon were braided separately using the Needleless Grasping Suture Technique[7] (Fig. 1B).

2.2.3 Separated v/s conjoined grafts

Both ends of each tendon were stitched separately or together using the whipstitch technique with four loops (Fig. 1C and D).

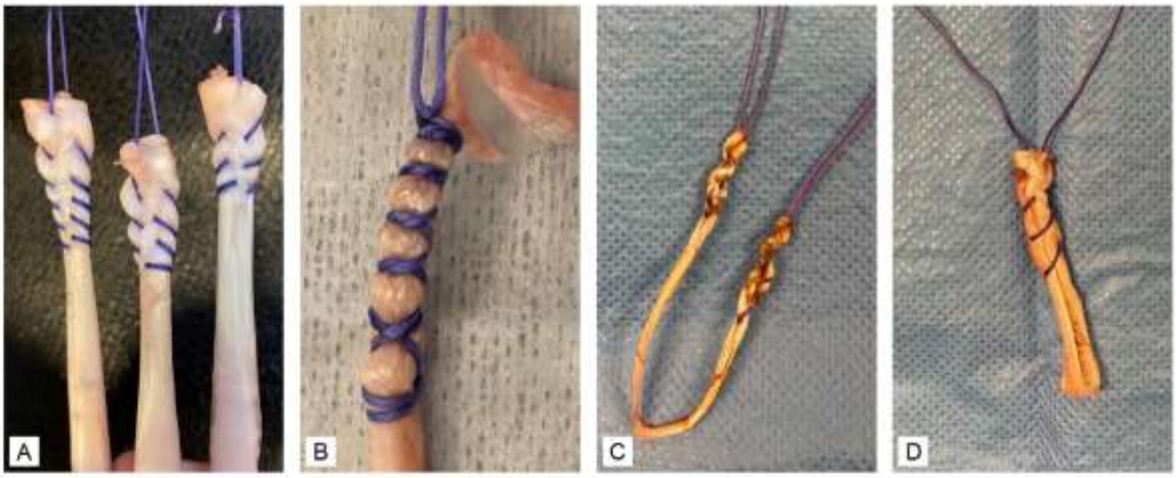

Fig. 1. A - Separated Whipstitch Grafts (3,4,5 loops); B - Braided Graft; C - Separated Whipstitch Grafts; D - Conjoined Whipstitch Grafts

2.3 Testing

The grafts were secured to the grips of the Zwick-Roell Z020 (Zwick-Roell GmbH Co. KG, Ulm, Germany) testing machine (Fig. 2). Each graft was preloaded with 10N and its diameter was measured using an electronic caliper. The grafts were tested for force at failure and type of failure (tendon failure v/s suture thread failure). The constant rate of extension between cross heads was 20 mm/min. The data was collected using TestXpert III - V1.7 (Zwick-Roell GmbH Co. KG, Ulm, Germany) and the results are given in Newtons (N) as a mean.

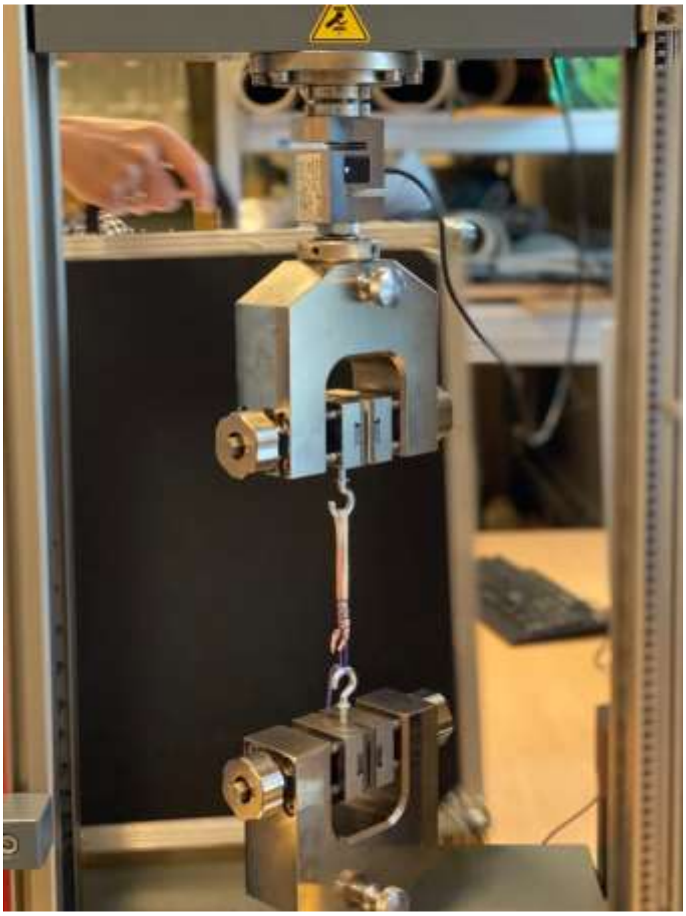

Fig. 2. Graft construct installed onto Zwick Roell Z020 testing machine

2.4 Statistics and data analysis

All data were collected in Excel 2019 (Microsoft Corporation, Redmond, WA, USA). The Statistica PL v. 13 was used for statistical analysis. The level of significance was $p<0,05$.

3. Results

3.1 Tendon diameters

There were no statistically significant differences between tendon diameters in groups 2 and 3

(*P* = 0,73; ANOVA) (Tab.1).

Tab. 1

Diameter (mm)	**Whipstitch - 3 loops**	**Whipstitch - 4 loops**	**Whipstitch - 5 loops**	**Braiding**
mean	5,17± 1,28	5,014± 2,07	4,97± 1,99	4,89± 1,57
max	7	9	8	7,71
min	3,2	2,8	2,8	2,8

Table 1: Difference in diameter (mm) between samples

P>0,05 (*P*=0,73)

Abbreviation: ±- standard deviation

3.2 Mechanisms of failure

There were two main types of mechanisms of failure - tendon tear (Fig. 3A) and suture tear (Fig. 3B). These types were preceded by linear increase in load until failure (Fig. 4A). In the case of braiding, the suture began to slip off (Fig. 3C) at a significant load before the tendon or suture rupture, resulting in an irregular chart appearance (Fig. 4B). Detailed data is available in the table (Tab. 2).

Tab. 2

Mechanism of failure/ No. of samples	**Whipstitch 3 loops**	**Whipstitch 4 loops**	**Whipstitch 5 loops**	**Braiding**
A	8	5	2	4
B	2	5	8	6
Slip off	0	0	0	5
No. of samples	10	10	10	10

Table 2: Mechanism of failure

Abbreviation: ±- standard deviation, A- tendon tear, B- suture tear

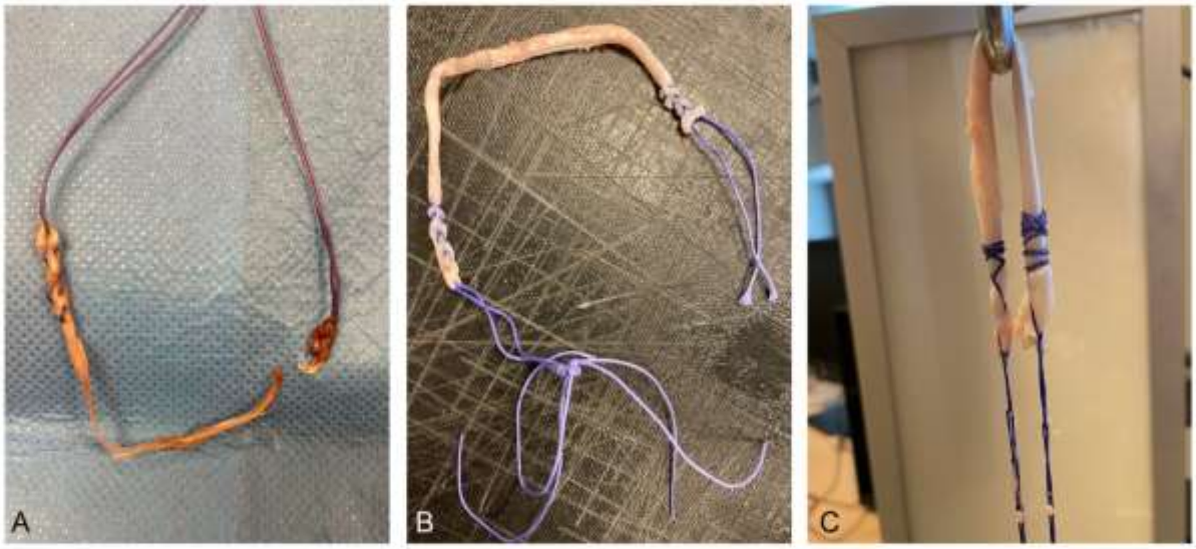

Fig. 3. Types of failure mechanism: A - tendon tear, B - suture tear, C - braided suture sliding off

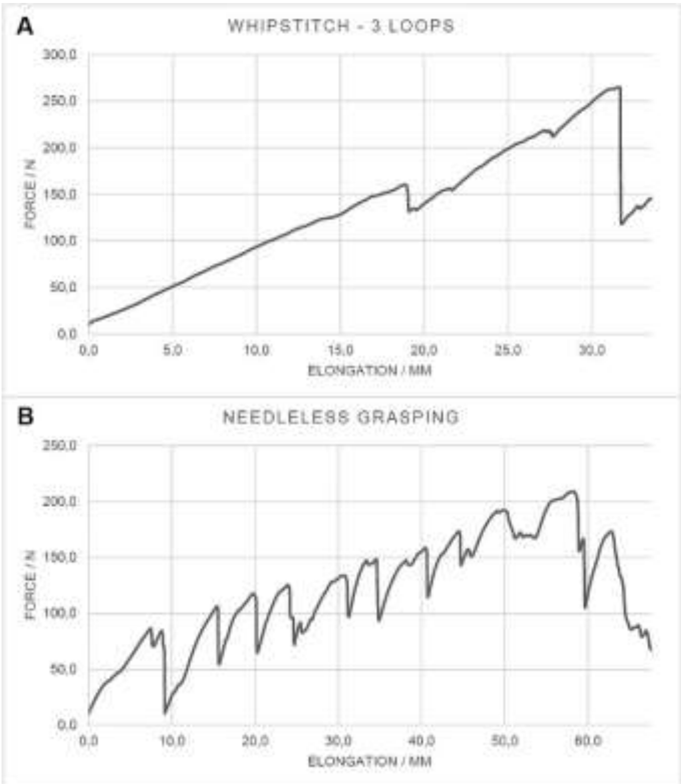

Fig. 4. A - Example of graph showing whipstitched graft tensioning until rupture. B - Example of graph showing braided graft tensioning until rupture.

3.3.1 Group 1

In group 1, the whipstitch with 3 loops had a force at failure of 185 N, while 4 loops achieved 241 N, and 5 loops had 254 N (Tab. 4). There was a statistically significant difference between the load at failure in the 3 loops vs 5 loops whipstitch. No statistically significant difference was found in the load to failure between the other groups.

3.3.2 Group 2

In group 2, the mean force at failure for braided grafts was 209 N, which was not statistically significantly different from the load at failure of grafts from group 3.

3.3.3 Group 3

In Group 3, the load at failure of the whipstitch of each tendon ends individually was compared to a whipstitch of both tendon ends folded together (Tab. 3). There was no statistically significant difference in tendon diameters between the separated whipstitch and conjoined grafts (P = 0,57, Mann Whitney U test). There was a statistically significant difference in load at failure between the separated whipstitch and conjoined grafts (P<0,001, Mann Whitney U test). The load at failure was greater for separated whipstitch grafts than for conjoined grafts (261 N v/s 152 N). In all cases the thread was the site of rupture.

All specimens in the study survived the minimal tensile load of 80 N, as suggested for optimal anterior cruciate ligament (ACL) graft tensioning.

Tab. 3

	Separated		**Conjoined**	
	Diameter (mm)	**Load (N)**	**Diameter (mm)**	**Load (N)**
mean	3,63± 1,44	261± 44,47	3,77± 2,25	151,89± 11,65
max	7	316	9,22	169
min	2	169	1,48	134

Table 3: Load at failure (N) and Diameter (mm) - whipstitch v/s conjoined grafts

Mann -Whitney U - Diameter P=0,57, Load P<0,001

Abbreviation: ±- standard deviation

Tab. 4

Load (N)	Whipstitch - 3 loops	Whipstitch - 4 loops	Whipstitch - 5 loops	Braiding
mean	174,7±74,34	220,6± 80,48	253,78± 44,85	209± 25,48
max	312	315	298	257
min	93	125	169	173

Table 4: Difference in load at failure (N) between samples

P>0,05 except 3 loops v/s 5 loops (*P*=0,017)

Abbreviation: ±- standard deviation

4. Discussion

4.1. Graft Preparation Methods

An anterior cruciate ligament (ACL) tear stands out as a prevalent injury in sports. Although the primary repair of the ligament remains a potential treatment method, ACL reconstruction has emerged as the prevailing standard of care[1,2].

Since the inception of anterior cruciate ligament reconstructive surgeries, several steps have been taken to optimize the procedure. The evidence indicates that employing allografts may increase the risk of failure, therefore autografts are a standard graft source[8–10].

While whipstitching the graft at the preparation stage is a current standard, several modifications of this method exist[11,12]. Needleless grasping technique was introduced in order to reduce the time, cost, and risk of needlestick injuries associated with graft preparation[13].

In 2022 Fang et al. conducted a similar study evaluating elongation after tensile loading on two graft models: a whipstitched tendon (with 3 loops), and one sutured with needleless grasping technique[7]. The grafts were subjected to a tensile load of 100N for 1 minute, and the load-elongation curve of the grafts was recorded. The study found no statistically significant difference in elongation between the two groups. The study also measured the difference in graft preparation time between the two methods. Graft preparation time in the needleless group was significantly shorter (mean 19.8 ±4.4s) than of the conventional whipstitch method – 52.7±12.7s. This difference appears to be a significant time advantage in the context of the number of ACL reconstructions performed daily worldwide.

However, this study tested a suturing technique with a fixed number of suture throws. Hong et al. conducted a biomechanical study similar to the current study, comparing different methods of graft end suturing and dividing them into groups based on the number of suture throws (loops)[14]. In their work, the elongation of the tendons after application of tensile force and the load to failure were measured. The suturing methods used were the Krackow stitch, the locking SpeedWhip and the modified finger trap (similar to the needleless technique used in this study). Each method was also divided into groups of 3, 5 and 7 suture throws (loops, passes). In their study, there was no significant difference in failure loads between the 3, 5 and 7-throw groups for any of the methods tested, nor was there a significant difference between any of the suturing methods for any number of suture throws. The current study reported a significant difference between whipstitch 3 loops (throws) and whipstitch 5 loops (throws) [*3.3.1*]. The study by Hong et al. also found no significant differences in elongation after cyclic loading.

Similar results to ours on suturing both tendon ends were obtained by Theopold et al. in their biomechanical study on porcine tendons[15]. They compared the load to failure of two preparation methods. First group: three suture technique - where both ends of the tendon are sutured separately, with a third suture looped around the midpoint of the tendon to form a four-strand graft. Second group: both ends sutured together with a single Krackow suture (4 throws), then the free ends of the suture were pulled through the formed circle to form a four-strand graft. Similar to this study [*3.3.3*], load-to-failure was significantly higher in tendons with separately sutured ends (711 N±91 N) compared to the single suture group (347 N±24 N, *P*=0.0001). However, both mean (as well as minimum) failure loads exceed the tensile forces that can be expected intraoperatively and therefore the method can be considered safe to use.

4.2. Graft Tensioning

There is no consensus on the ideal graft tension. In our study a target of withstanding minimum 80N of tensile force was set, based on the available biomechanical evidence, which will be discussed later.

Yasuda et al. conducted a prospective study on 70 patients with isolated ACL tears, dividing them into three groups based on the initial tension of the graft during reconstruction surgery[4]. The grafts used were doubled autogenous hamstring tendons connected with polyester tape. The tension levels were set at 20N, 40N, and 80N for Group I, Group II, and Group III, respectively. Postoperative side-to-side anterior laxity was measured at 30° of flexion. A statistically significant difference was found between the 20N and 80N tensioned groups, and a significant correlation was observed between the tension set on the graft and the degree of postoperative laxity. The authors advocate using of tension of approximately 80N in order to reduce postoperative side-to-side knee laxity.

Aforementioned study was taken into account in a 2009 systematic review by Arneja et al [5]. The review draws its conclusion from 5 articles, showing evidence of a trend suggesting 80N of tension to be the most effective amount for a hamstring-polyester graft. However, no clear trend was found for a specific tension to use for a semitendinosus-gracilis or patellar tendon graft. The authors state that they were unable to recommend a standard value for a 4-strand graft.

In a 2002 paper, Heis et al. call for tissue-specific tensioning. The authors recommend 5 pounds (22.24N) of tension for a patellar tendon graft and 15 pounds (66.72N) of tension with each bundle tensioned individually for a hamstring graft[16].

Another systematic review on this topic by Kirwan et al concluded that 78.5 to 90N is the preferred initial graft tension to minimise side-to-side displacement in anterior knee laxity[6]. However, the authors note that there is no evidence to suggest that any specific tension value improves patient knee function.

The most recent study compared the effects of different tensions in 60 double-bundle ACL reconstructions[17]. The tensions compared were 20N, 25N and 30N for the anteromedial bundle and posterolateral bundle separately. The results showed no significant difference in anterior knee laxity between the 30N and 25N tension groups. Additionally, there was no positive pivot shift observed in these groups. Koga et al. concluded that initial graft tension in double-banded ACL grafts can be as low as 25N (per 6mm in graft diameter). They advised against tensioning at 20N (per 6mm in graft diameter) and below due to the potential for residual pivot shift.

All samples in our study exceeded the recommended values mentioned earlier. Therefore, in theory, all methods could be used in real operating room conditions. However only whipstitched grafts have shown a consistent strength distribution during tensioning. This can cause problems when tensioning is done by hand without measuring devices while using needleless grasping technique. The surgeon may apply too much tension on the graft causing suture loops to “slip off”, as shown in this study [*3.2*].

4. 3. Limitations

Due to their high availability, low cost and ethical considerations, some biomechanical studies have been conducted in animal models[18,19]. Similarly, our study used porcine extensor digitorum tendons, a commonly used substitute for human semitendinosus graft in ACL reconstruction research[20,21]. In their biomechanical comparison, Omar et al. found that the elongation during cyclic loading was similar for both tendons. However, the load to failure of the porcine tendon was significantly lower than that of the human tendon[22]. The lower tensile strength of porcine tendon is not a significant aspect in our results. Since all models withstood minimal 80N of force applied [*3.3.2*], it is expected that human tendon would also withstand this. Nevertheless, our use of an animal model is a limitation that should be noted.

The tendons were sutured by three medical students, which may have resulted in variations in the quality of the constructs. Furthermore, tendons were harvested from three pigs, with two posterior legs from each, which could also lead to differences in quality. However, the grafts were randomly divided, so these limitations should not affect our study.

4.4. Clinical implications and future outlook

Recognizing the frequency and significance of ACL reconstruction procedures[2], we encourage to use our study's findings as a valuable guide to optimize this process. As the ultimate load to failure was greater than 80N for all techniques used, theoretically all techniques can be used for primary ACL graft preparation. Surgeons can therefore choose the graft preparation technique that best suits their preferences and time constraints. This will help to reduce the time spent on graft preparation and consequently the overall duration of surgery. As a result, costs and the number of procedure-related complications are expected to decrease. However, as shown in our study, tendons prepared using the needleless grasping technique, were more prone to suture sliding off at higher tensile loads. This unpredictability in force output has to be taken into account when choosing a technique.

A similar study that utilizes human tendons would be needed to verify the tensile forces found in this study.

5. Conclusion

All of the techniques utilized in our study exhibited ultimate failure loads exceeding 80N, with a minimum force at failure of 93N observed in a 3.2mm triple whipstitched tendon. Consequently, theoretically, all techniques are viable for primary ACL graft preparation. While the braiding technique meets the strength requirements for strand/tendon connection, its susceptibility to suture slippage raises concerns about consistent strength distribution during tensioning. Conversely, the whipstitch with five loops emerged as the strongest technique tested. It is important to note that the strength of this model is primarily dependent on suture strength rather than tendon strength.

Acknowledgements

Author MC is designated as a co-first author of this paper, sharing equal credit for the work presented.

This research was funded in whole by National Science Center (NCN) in Cracow, Poland, within Grant No. 2022/47/D/ST8/02713.

Funding

This research was funded in whole by National Science Center (NCN) in Cracow, Poland, within Grant No. 2022/47/D/ST8/02713. Funding source played no role in the study design; collection, analysis and interpretation of data; writing of the report; or in the decision to submit the article for publication.

Declaration of interests

none

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