Comparison of the mechanical properties and mechanical damages to tendon tissue in three suspensory fixation techniques

Toshiya Kano [a], Masakazu Ishikawa [b, *], Yuki Ogawa [c], Hiroyuki Akebono [c], Atsushi Sugeta [c], Masato Ohmi [d], Goki Kamei [a], Atsuo Nakamae [a], Nobuo Adachi [a]

[a] Dept. of Orthopaedic Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Dept. of Artificial Joints and Biomaterials, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[c] Lab. of Strength and Fracture of Materials, Mechanical Science and Engineering, Hiroshima University, Hiroshima, Japan
[d] Course of Health Science, Graduate School of Medicine, Osaka University, Osaka, Japan

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ABSTRACT

Background: Anterior cruciate ligament (ACL) injury is the most common traumatic injury to the knee joint. Suspensory fixation has become popular in ACL reconstruction because of its high primary stability, less invasiveness, and surgical convenience. There are two common types of suspensory fixation devices: those with fixed-length and those with adjustable-length loops. Owing to structural differences and differences in initial tensioning techniques, it is expected that mechanical property and damage to the tendons will vary from device to device; however, no literature has examined this so far. The main purpose of this study was to evaluate the damage caused to the tendon by three different suspensory fixation devices. An effective mechanical test was carried out as a prerequisite.

Methods: First, the mechanical properties of simple loop device (SLD) as fixed-length loop device, first-generation, and second-generation adjustable devices (AD1 and AD2) as adjustable-length loop devices were tested (isolated device testing). Second, each device was tested using bovine extensor tendons (specimen testing). Cyclic testing included 2000 cycles; the devices were subsequently displaced until failure, and the ultimate tensile strength was determined using isolated device testing. Six samples of 3 devices were used in each testing experiment. After specimen testing, the surface structure of the tendon was evaluated quantitatively using optical coherence tomography (OCT) and our original histological scoring system.

Results: During isolated device testing, SLD demonstrated the least cyclic displacement, followed by AD1 and AD2. The highest ultimate tensile strength was observed in AD2, followed by SLD and AD1. In specimen testing, the least cyclic displacement was observed in SLD, followed by AD1 and AD2. Histologically, AD1 demonstrated a significantly lower score, with damaged surface morphology, than SLD and AD2. OCT values were significantly higher, with a more disturbing tendon surface structure, in AD1 than in SLD and AD2.

Conclusions: The first-generation adjustable loop device exhibited greatest graft tissue damage at the suspensory site in a clinically relevant setting. The thinner adjustable loop mechanism may have elevated graft damage by frictional stresses during loop adjustment or by repetitive tensioning stresses.

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1. Introduction

Anterior cruciate ligament (ACL) injury is the most common traumatic injury to the knee joint. ACL reconstruction is the gold standard in the treatment of ACL injury.[1–3] The success of ACL reconstruction is highly dependent on several factors such as graft selection, tunnel positioning, and the mechanical properties of the

fixation device. Trojani et al. investigated the cause of anterior cruciate ligament reconstruction failure in a multicentre retrospective study and reported that fixation system failure accounted for 5% of the failure.[4] Recently, suspensory fixation has become popular in ACL reconstruction because of its high primary stability, relatively low invasiveness, and surgical convenience.[5–7] Currently, there are two common types of suspensory fixation devices: fixed-length and adjustable-length loops. Although an adjustable-length loop has the advantages of bone preservation and a greater contact area between the grafted tendon and bone tunnel, its disadvantage is loop lengthening after cyclic loading.[8,9] However, few studies have shown significant differences in their clinical effect, and therefore the optimal femoral fixation device remains controversial.

Simple loop device (SLD) is a clinically widely used fixed-length loop device with a continuous-loop polyester suture. The first-generation adjustable fixation device (AD1) is an adjustable-loop device with a Chinese finger trap mechanism and became popular in ACL reconstruction. This AD1 includes an ultrahigh molecular weight polyethylene and polyester suture running through the button, and its traction part has a simple structure in which thin loops are combined in the form of a chain. In comparison to SLD, a possible disadvantage of AD1 is its thin loop which potentially causes friction between the grafted tendon and the loop during length adjustment and more stress on tendon. To improve these points, the second-generation adjustable device (AD2) was introduced in clinical practice. The AD2 is composed of ultrahigh molecular weight polyethylene and a unique cradle shape. In addition to the relatively thick structure of the cradle itself, the thread moves inside the cradle during loop adjustment, resulting in minimal friction with the graft tendon, which may improve the level of damage to the graft tendon. We suspect that friction stress during loop adjustment and graft suspension over a smaller contact area could result in increased mechanical stress on the graft, thus, resulting in graft failure. However, no studies have evaluated the effect of the suspensory fixation device on graft damage.

The main purpose of this study was to clarify how structural differences in the loop mechanism affect mechanical damages to tendon tissue. The mechanical strength and loop lengthening of the three suspensory devices (SLD, AD1, and AD2) were investigated to ensure that a valid mechanical testing was performed. We hypothesized that 1) devices that induce friction stress on soft tissue with the adjustable mechanism would cause more damage to the graft, and 2) devices with smaller loop diameters would have a potential to cause more damage to the graft.

2. Materials and methods

The three suspensory fixation devices tested in this study were SLD 20 mm (EndoButton™ CL, Smith & Nephew, London, UK), AD1 (TightRope®, Arthrex, Naples, FL, USA), and AD2 (UltraButton™ CL, Smith & Nephew, London, UK) (Fig. 1A, B, C). The 20-mm loop length used in this study is a particularly commonly used size for ACL reconstruction and has also been frequently used in previous reports.[8,9] Each device was tested with two separate and independent protocols, and all the devices used in each protocol were brand new. First, the biomechanical properties of each device were tested (isolated device testing). Second, each device was tested on bovine extensor tendons to mimic actual clinical grafting (specimen testing). The number of samples for each device was 6 in both protocols. The tests were performed using a Shimadzu dynamic fatigue testing machine (SERVOPULSER, Shimadzu Corporation, Kyoto, Japan). The jig used in this experiment was a custom-made apparatus according to previous report (NISSIN SEIKI, Hiroshima, Japan).[10]

2.1. Set up for isolated device testing

To evaluate the properties of the devices without the influence of the biological environment, initial testing was performed using a custom-made apparatus that consisted of a steel plate as the cortex and a steel rod, which applied force to the loop of each fixation device (Fig. 1D). The thickness of the central part of the loop in each device (N = 6) was measured to clarify the characteristic shape of loop in each type of suspensory device using a digital calliper (Shinwa Rules Co, Niigata, Japan). The measurement was performed under 50 N of tension to the loop using a 6 mm diameter steel rod.

Tunnel diameters were 4.0 mm for AD1 and 4.5 mm for SLD and AD2. Each tunnel diameter was chosen according to the size of buttons. The thickness of the plate (8 mm) was set not to impinge with the loop of devices and not to distorted by the force of 2000 N. The cortical buttons were inserted through a hole into the steel plate, and the steel rod was inserted into the SLD loop. The distance from the upper edge of the plate to the lower edge of the rod was set to 20 mm. AD1 and AD2 were set up similarly, and maximum tension was manually applied to reduce the loop size from 35 mm to 20 mm using a dedicated recommended device to reproduce the surgical procedure. Both devices were completely tightened before testing commenced.

2.2. Set up for specimen testing

The lower legs of bovines slaughtered for meat were purchased, and three extensor tendons were excised from each leg following previous reports.[11,12] The harvested tendons were cleared of adherent muscle fibres and surrounding soft-tissue, and their diameters were measured using a diameter measurement tool (Smith and Nephew, Andover, USA). Tendons of similar diameters were then selected to eliminate the influence of individual differences. Selected tendons were cut to a length of 150 mm before being wrapped and stored at −20 °C until use following a previous report.[13] For specimen testing, tendons were thawed by immersing them in phosphate buffer solution (PBS) on the testing day. Similar to isolated device testing, the cortical button was placed on the steel plate, and the extensor tendon was suspended on the loop of the device and secured with a dedicated clamp (Fig. 1E).[10] The testing machine was set such that the distance from the plate to the clamp was 50 mm with the SLD loop and the extensor tendon under tension. AD1 and AD2 were tested in similar conditions, and maximum tension was manually applied to reduce the loop size to from 35 mm to 20 mm using a dedicated recommended device for each device following the practical surgical procedure.

2.3. Testing protocols

A constant preload of 50 N was applied to the device or specimen for 30 s, and the displacement was measured. This measurement was described as preload displacement. The point at which the displacement reached a plateau was set as the zero-displacement point in the next cyclic testing, which was performed by applying sinusoidal loading of 50−250 N at a frequency of 2 Hz for 2000 cycles. We set these testing protocols, simulating walking movements, following previous reports.[14–16] Data were recorded every two cycles, and the elongation from the zero-displacement point at the time of the 50 N load at 2000 cycles was defined as cyclic displacement. For the isolated device testing protocol, the devices were further displaced at 1 mm/s until failure. The first visible drop in load was described as the ultimate tensile strength. After cyclic loading with specimen testing, the bovine tendons were carefully removed from the apparatus for tissue evaluation.
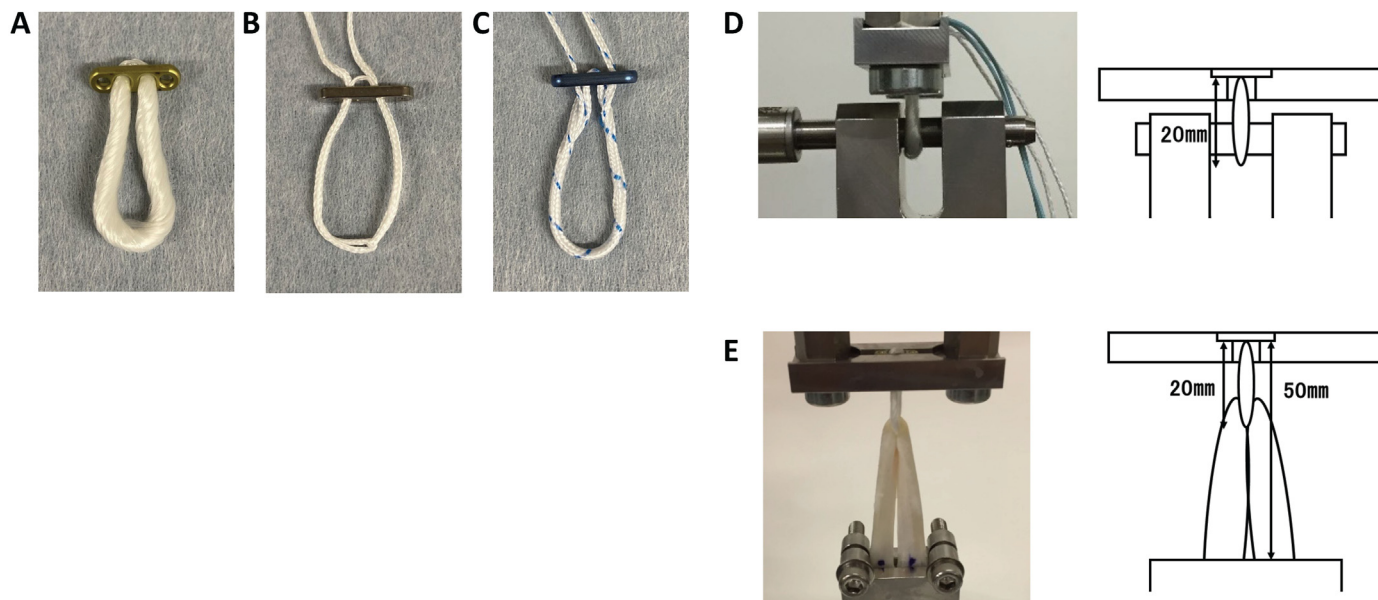
Fig. 1. Images of suspensory devices and testing devices.
A. Simple loop device (SLD) with a 20-mm continuous-loop suture. **B.** The first-generation adjustable device (AD1). **C.** Second-generation adjustable device (AD2). **D.** Set up for isolated device testing. Each device was suspended using a steel rod with diameter of 6 mm. The length of loop was adjusted to 20 mm. **E.** Set up for specimen testing. Bovine tendon was suspended on each device and held with tendon clamp. The length of loop was adjusted to 20 mm following isolated device testing and the distance from the plate to the clamp was set to 50 mm.

2.4. Optical coherence tomography

After specimen testing, the tissue was fixed in PBS containing 4% paraformaldehyde for 24 h. Fixed samples were washed with PBS and stored at 4 °C until analysis. In addition, samples of normal tendon tissue (N = 6) were prepared with the same procedure as a control group. To quantitatively evaluate tendon fibre irregularity, two-dimensional (2-D) optical coherence tomography (OCT) images were obtained. OCT measurements of all bovine tendon samples were performed using a spectral-domain OCT device (Thorlabs, NJ, USA) (Fig. 2A). The image taken at the centre of the suspension part was acquired on the short axis in the range of 200 μm. The field of view in each sample was 4.0 × 3.0 mm (width × depth), generating a 2-D data matrix of 400 × 512 pixels (Fig. 2B). The 2-D B-scan thus obtained was used for further analysis. For quantitative image analysis of OCT data, custom-made software was utilized with MATLAB (MathWorks Inc., MA, USA). To examine the surface area in contact with the suspension device, we selected a region of interest (ROI) 200 × 120 μm (width × depth) in each sample. This ROI was divided into three zones from top to bottom: the superficial zone (SZ), middle zone (MZ), and deep zone (DZ) (Fig. 2C). Using custom-made software, we obtained mean OCT values of the reflection intensity of tissue samples using values from each pixel. Increased OCT values represent a rough structure due to surface damage. The mean OCT values of each zone was obtained and compared between the devices.

2.5. Histological evaluation

After OCT imaging, the bovine tendon was cut to 20 mm for sectioning, including the site where the suspensory fixation device was in contact. The samples were embedded in paraffin and sectioned at a thickness of 4.5 μm, deparaffinized in xylene, dehydrated using an ethanol series, and stained with haematoxylin-eosin. The surface structure of the suspension part of the tendon was then observed microscopically. Each section was scored using a scale of 0−3 points according to the surface structure in a blinded manner (0: extremely smooth; 1: relatively smooth; 2: rough and irregular; 3: extremely rough and irregular) (Fig. 3). Intra- and interobserver reliabilities for the histological scoring were assessed by two independent observers. For intra-observer reliabilities, scoring was performed on two occasions separated by 4 weeks. Reliabilities for measurements of interval data were assessed with the intraclass correlation coefficients (ICCs). The ICC values were analysed using SPSS software (SPSS, Inc., IL, USA). The intra- and interobserver ICCs for histological scoring were both excellent (intraobserver: 0.951, 95% confidence interval (CI) [0.88, 0.98], interobserver: 0.860, 95% CI [0.67,0.95]).

2.6. Statistical analysis

All values are expressed as mean ± standard deviation. We performed a statistical analysis of the thickness of the central part of the loop, the cyclic displacement, tensile strength, tendon diameter, OCT values, and histological score between the three groups. Kruskal−Wallis test with Dunn's post hoc test was carried out to compare the three groups. P values < 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad PRISM (GraphPad Software, Inc., CA, USA).

In the statistical power analysis, it was estimated that the sample size of this study (six devices for each group) was adequate to achieve a statistical power of 99% with less than a 5% probability of a type I error and a 95% confidence interval.[17−19]

3. Results

3.1. Isolated device testing

The thickness of the central part of the loop was significantly thicker in SLD (2.88 ± 0.19 mm) than that in AD1 (1.03 ± 0.08 mm, p < 0.001) and AD2 (2.02 ± 0.03 mm, p < 0.001), and AD2 was significantly thicker than AD1 (p < 0.001).

The cyclic displacement after 2000 cycles in SLD
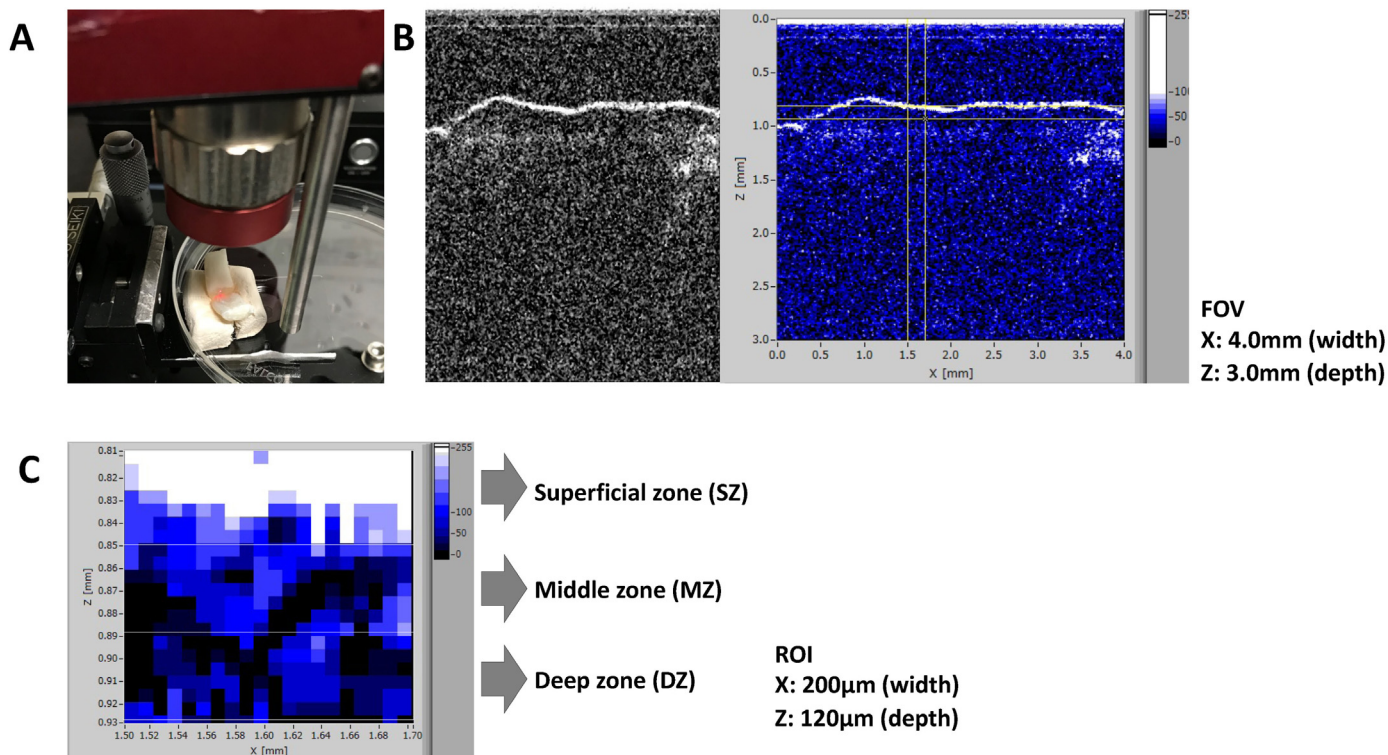
Fig. 2. Optical coherence tomography.
A. Apparatus and specimen setting for image acquisition of optical coherence tomography (OCT). B. Acquired raw OCT image and field of view (FOV) (left), 4.0 mm in width and 3.0 in depth, and selected area of region of interest (ROI) for analysis (right). C. Three zones of region of interest (ROI), 200 μm in width and 120 μm in depth, with 400 x 512 pixels for analysis of OCT value. Higher OCT value was presented whiter colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
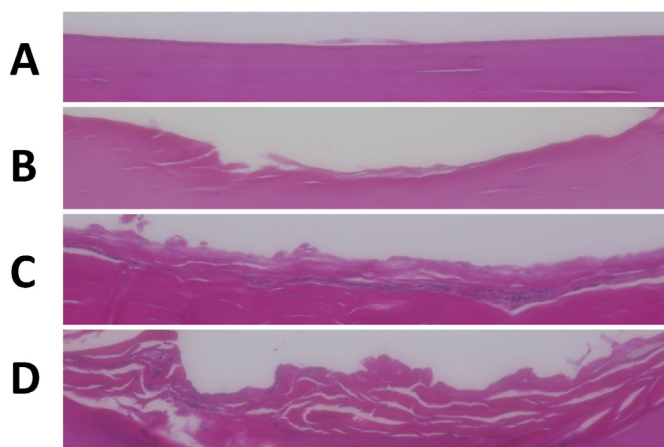
Fig. 3. Histological grading system.
A. Extremely smooth, score 0. B. relatively smooth, score 1. C. rough and irregular, score 2. D. Extremely rough and irregular, score 3.

(0.57 ± 0.10 mm) was significantly smaller than those of AD1 (1.14 ± 0.13 mm, p = 0.003) and AD2 (1.21 ± 0.29 mm, p = 0.001), while no significant difference was identified between AD1 and AD2 (p > 0.99) (Fig. 4A).

The ultimate tensile strength in AD1 (836 ± 40 N) was significantly lower than those in SLD (1419 ± 122 N, p = 0.015) and AD2 (1682 ± 151 N, p = 0.007), while no significant difference was identified between SLD and AD2 (p = 0.091). All failures occurred at the centre part of the loop in both SLD and AD1, whereas in AD2, all buttons were broken with bended manner at the part of connection with the loop.

3.2. Specimen testing

Before specimen testing, we confirmed that there was no significant difference in tendon diameter between the three groups (SLD: 9.75 ± 0.69 mm; AD1: 9.67 ± 0.23 mm; and AD2: 9.41 ± 0.67, p > 0.99). The cyclic displacement after 2000 cycles in SLD (1.77 ± 0.20 mm) was significantly smaller than those of AD1 (2.47 ± 0.31 mm, p = 0.019) and AD2 (2.77 ± 0.56 mm, p = 0.001), while no significant difference was identified between AD1 and AD2 (p = 0.599) (Fig. 4B).

3.3. Optical coherence tomography

OCT values were significantly higher in each zone in AD1 than in SLD and AD2 (SZ: p < 0.001, MZ: p < 0.001, DZ: p < 0.001, in both comparisons). In the superficial zone, AD2 had significantly higher values compared to SLD (p < 0.001). On the other hand, in the middle and deep zones, SLD had significantly higher values than AD2 (MZ: p < 0.001, DZ: p < 0.001). In comparison with normal tendon tissue, SLD had significantly higher OCT values in the middle and deep zones (SZ: p > 0.99, MZ: p < 0.001, DZ: p < 0.001), AD1 had significantly higher values in All zones (SZ: p < 0.001, MZ: p < 0.001, DZ: p < 0.001), and AD2 had significantly higher value only in the superficial zone (SZ: p < 0.001, MZ: p > 0.99, DZ: p = 0.103) (Fig. 5).
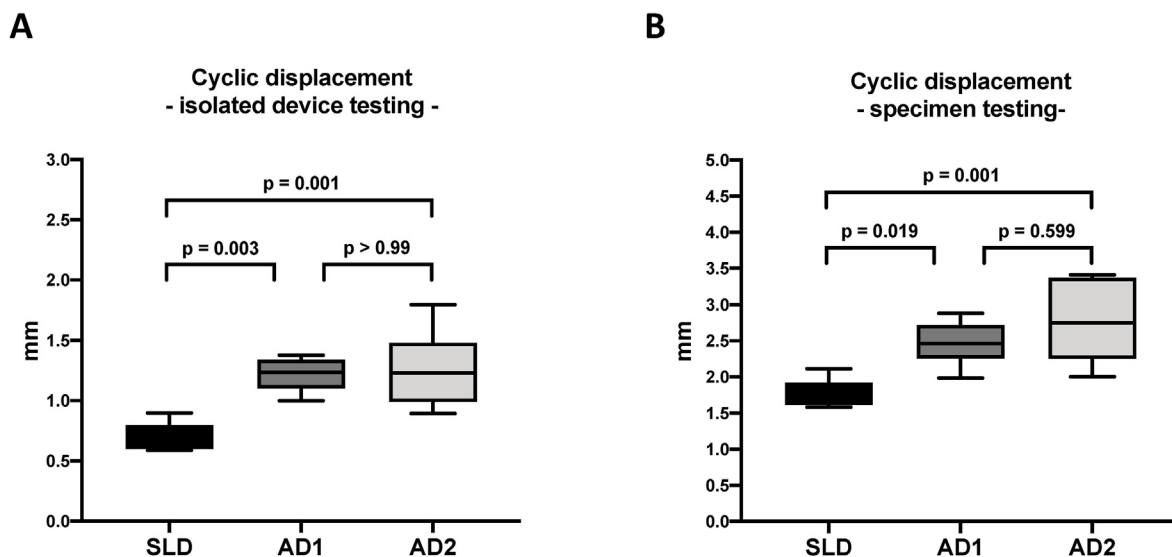
Fig. 4. Isolated device and specimen mechanical testing.
A. Mean displacement after 2000 cycles of load in isolated device testing. B. Mean displacement after 2000 cycles of load in specimen testing.
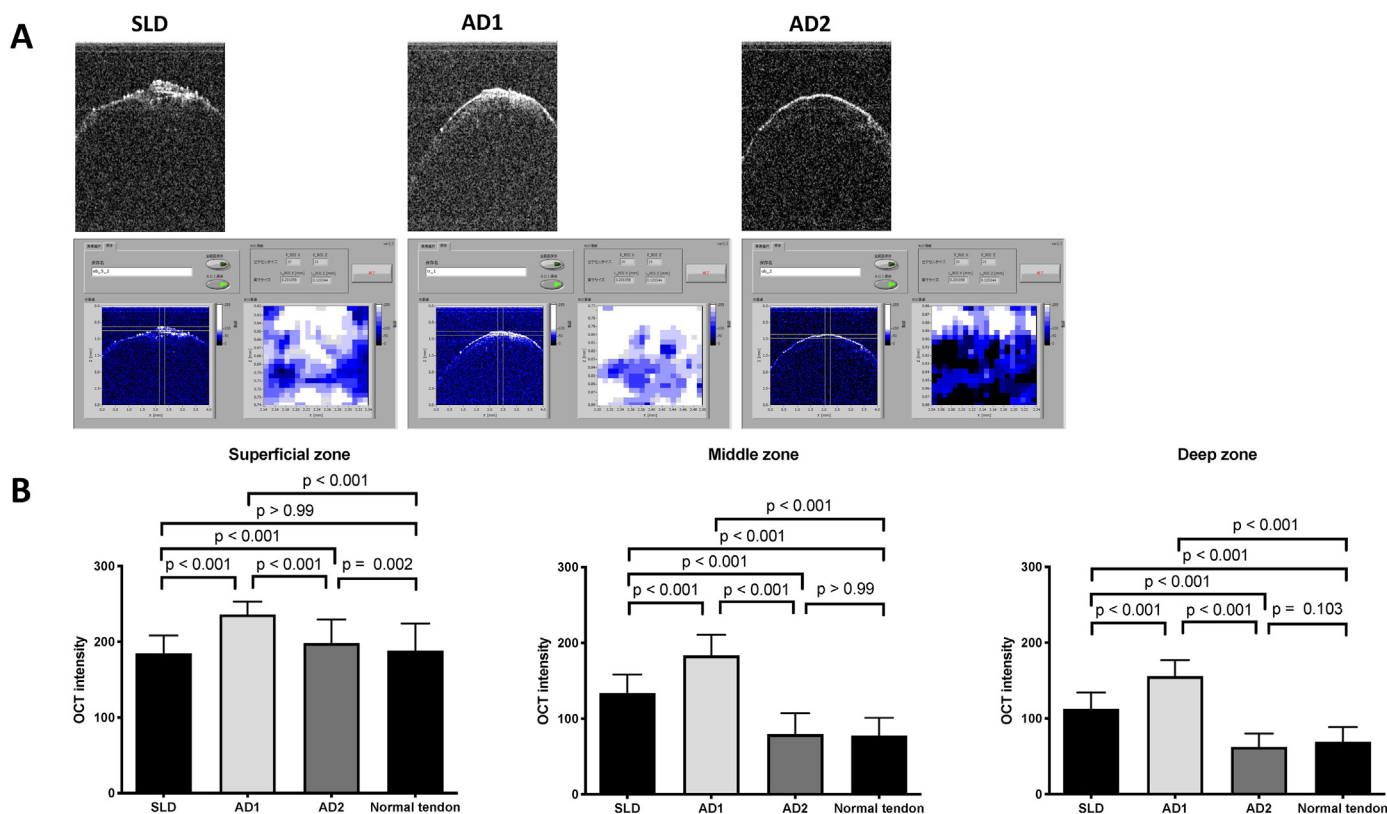

Fig. 5. Image analysis and comparison of optical coherent tomography data among groups.
A. Representative optical coherence tomography (OCT) images of each group. SLD, simple loop device; AD1, first-generation adjustable fixation device, AD2, second-generation adjustable fixation device. B. Comparison of OCT values in each zone of the surface between the groups.

3.4. Gross observation and histological evaluation

Macroscopic observation revealed a depression of the tendon surface according to the respective diameter at the site contacting with the loop (Fig. 6A). Histological findings demonstrated extremely rough and irregular surface in AD1 compared to SLR and AD2 samples (Fig. 6B). Histological scores were significantly higher for AD1 (2.5 ± 0.8) than for SLD (0.7 ± 0.7, p = 0.020) and AD2 (0.8 ± 0.7, p = 0.048), while no significant difference was identified between SLD and AD2 (p > 0.99) (Fig. 6C).
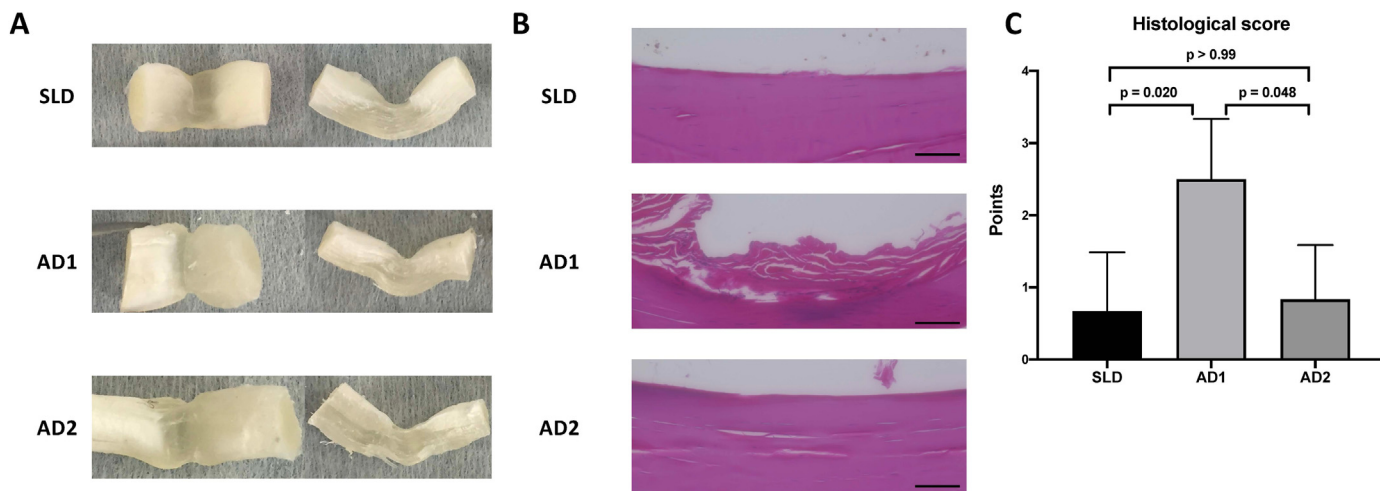
Fig. 6. Gross observation and Histological evaluation.
A. Macroscopic findings: left is frontal view of the area in contact with the suspension device, right is cross-sectional view from the side. B. Microscopic findings: Haematoxylin-eosin staining (original magnification X200). Scale bar represents 100 μm. C. Comparison of histological scores between the groups.

4. Discussion

The most important finding of this biomechanical and histological study is that the damage to the tendon surface differed with each suspensory fixation device. The surface structure of the graft on the suspension part showed severer damage from the AD1 compared with SLD and AD2. These results were consistent with our original hypothesis.

In the field of orthopaedics, OCT was introduced first for the evaluation of articular cartilage.[20] Martin et al. reported that OCT holds promise as a method for assessing the microstructural organization of the tendons and ligaments.[21] OCT has also been used to assess damage to the tendon tissue because heavy damage to the connective tissue leads to the loss of an ordered structure such as fibre disruption and tearing, and consequently changes the tissue's optical properties.[22] In this study, the use of OCT allowed for a quantitative assessment of the surface of the tendon where it is in contact with the suspensory fixation device. Moreover, the histological findings of the surface of the tendon revealed a similar trend to OCT findings. Particularly, significant surface structural changes were observed with AD1 in both OCT and histological evaluations.

Since the pressure per unit area is inversely proportional to the thickness, the use of thin loops will increase the force exerted on the suspensory part of the tendon. As a concern at the outset, it was suggested that AD1 with an adjustable mechanism and a small diameter could affect the surface structure of the tendon at the contact area. In recent mechanical testing study, Dias et al. investigated the effect of the contact force, pressure and area of suture materials on the tendon. They concluded that suture tape, larger contact area, has smaller pressure over the tendon compared to the suture wire, smaller contact area, and this may lead a favourable perfusion environment for tendon healing.[23] Hence, the effects of an adjustable-length device on the graft surface may affect graft maturation or cause the failure of ACL reconstruction due to poor perfusion environment, although further studies will be required to investigate this in vivo.

This study demonstrated that the fixed-length device (SLD) showed the least amount of lengthening during cyclic loading compared to adjustable-length devices (AD1 and AD2). However, the mechanical strength of AD2, an adjustable-length device, was greater than that of the other two devices. The results of this cyclic

displacement were similar to previous reports, confirming that a valid test was performed.[8,9]

The critical period for the performance of a fixation device to maintain joint stability is reported to be 6–12 weeks postoperatively before graft incorporation takes place.[24–26] One of the factors that determine postoperative fixation strength is the ultimate tensile strength. The highest ultimate tensile strength was observed with AD2, followed by that in SLD and AD1. It is estimated that graft fixation devices may withstand loads of at least 450–500 N during early rehabilitation.[27] Therefore, each of the devices used in this study was strong enough to bear this load.

The Chinese finger trap mechanism is a critical feature for allowing adjustable length; however, lengthening of the loop in the early phase could be a major problem associated with this device.[15,18,28] To secure the initial fixation, firm tensioning is necessary to avoid lengthening during surgery. AD1 has a simple structure; therefore, it is easy to apply enough tension during loop adjustment (Fig. 7A). In contrast, AD2 has three loops, making it difficult to apply tension to all the loops and, therefore, requires careful procedures. Additionally, since two loops are contained in the sheath of the Chinese finger trap, there is a gap between the sheath and loop; therefore, there is a possibility that the loop may loosen if it is not provided with enough tension (Fig. 7B). Thus, suture slippage into the sheath of the Chinese finger trap could be a critical reason for the difficulty in applying the tension as well. For the AD2 in particular, the low profile of the sheath for graft protection may affect lengthening compared with the simple Chinese finger trap mechanism.

There are several limitations to this current study. First, we tested only one condition for initial tension and cyclic load. The stress upon reconstructed tendon via a suspension device is varied during patient own daily activity. Therefore, it will be favourable to carry out different conditions as further studies. Second, the experiment was performed in dry conditions; a wet environment that simulates the in vivo joint might have a potential to demonstrate more precise data. Third, it is unclear whether it was cyclic loading or friction stress during loop length adjustment that affected tendon surface damage. Also, the effect of the damage to the tendon surface on the strength of the reconstructed ligament remains unclear. However, there is no study to investigate the graft damage due to suspensory fixation device. This study will provide

A   **B**
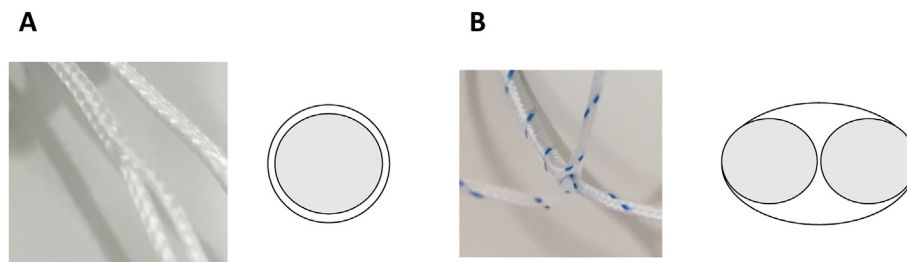
Fig. 7. Difference of Chinese finger trap mechanism between the first- and second adjustable devices.
Macroscopic image and schema of the first-generation adjustable device (AD1) (A) and second-generation adjustable device (AD2) (B).

new insight into how the surgeon select suspensory fixation device for more promising ACL reconstruction. To overcome these limitations, in vivo experiments with large animals and cadavers under various conditions will be needed in the future.

5. Conclusions

Among the three representative suspensory devices utilized in modern ACL reconstruction, the first-generation adjustable loop device exhibited greatest graft tissue damage at the suspensory site, after experimental cyclic loading in a clinically relevant setting. Potentially, the thinner adjustable loop mechanism may have elevated graft damage by repetitive tensioning stresses with higher contact force and pressure.

Author's contributions

TK performed biomechanical testing, histological evaluation, statistical analysis, and drafted the manuscript. MI conceived and designed the study, coordination, carried out image acquisition and analysis of optical coherence tomography and helped to draft the manuscript. YO, HA, and AS analyzed and supervised biomechanical testing. MO analyzed and supervised optical coherence tomography. GK and AN performed data analysis and interpretation. NA supervised the project and preformed proof reading the article. All authors read and approved the final manuscript.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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