

# The braking force to Control the Abnormal Tibial Translation Is a Critical Factor in Promoting the Spontaneous Healing of Complete Ruptured Anterior Cruciate Ligament in Mice Model

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**INTRODUCTION:** The anterior cruciate ligament (ACL) is essential to knee joint stability and movement. Once ACL injuries occur, the knee loses the proper bone kinematics during joint motion in daily activities. It has been considered that the ACL has low self-healing capacity because of the poor vascular supply and poor fibroblast activity of the injured ACL. Therefore, ACL reconstruction is generally recommended for ACL-injured patients. Conversely, several recent clinical studies have reported that ruptured ACL could heal spontaneously. However, there is insufficient knowledge of appropriate post-injury management of the knee joint to lead complete-ruptured ACL to spontaneous healing. Filbay et al. reported spontaneous ACL healing with the Cross Bracing Protocol, which constantly fixed the knee at 90 degrees in patients with ACL injuries. They showed that 90% of patients had evidence of healing on a three-month MRI. In our previous report using the rat model, we elucidated that the ACL could heal spontaneously due to only controlling abnormal anterior tibial translation (ATT) after ACL injury. Regarding mechanical stress, we should not immobilize the knee joint after ACL injury because appropriate mechanical loading promotes collagen remodeling during the ligament healing process. Thus, understanding the acceptable range of mechanical stress in the spontaneous healing response of the ACL is important for establishing non-surgical treatment and appropriate management of the injured ACL. In this study, we investigated the effect of different braking forces of the ATT on the healing response of the completely injured ACL. The aim of this study is to define the acceptable mechanical stresses during the ACL healing in this study.

**METHODS:** This study was approved by the Animal Research Committee of Saitama Prefectural University (approval number: 2021-9). We used 8-week-old male and female C57BL/6 mice (n=58). First, we underwent non-invasive ACL rupture in the left hindlimb under deep anesthesia. Then, we created the Controlled Anterior Tibial Translation (CATT) model to lead to the healing of the injured ACL. We made bone holes at the femoral condyle and proximal tibia; then a single nylon suture was threaded through the two holes to control the ATT. We used 3-0 and 4-0 nylon sutures to evaluate the effect of different braking forces of the ATT on the healing response of the ACL. Mice were sacrificed immediately after injury, at 4 and 8 weeks for each analysis. The right hindlimb was also used as Intact. Before creating the CATT models, we performed Failure and Cyclic testing and measured the mechanical properties of nylon sutures. After we collected all knee samples, we performed Anterior Drawer Testing to evaluate the braking force of the CATT knees and healed ACL knees at each time point. First, we measured the ATT of the CATT knees with nylon sutures. We then removed the nylon suture and measured without CATT again to evaluate the braking force of the healed ACL. In histology, we performed HE, Picrosirius Red (PsR), and Immunofluorescence (IF) staining. Grayscale images obtained from PsR-stained images were used to evaluate healed ACL maturity. In addition, we targeted type I collagen with IF staining to assess its localization on the healed ACL. In the statistical analysis, we analyzed for normality using the Shapiro-Wilk test and evaluated the homogeneity of variances with the Bartlett test. Then, we performed the Kruskal-Wallis test and the Steel-Dwass test to evaluate multiple comparisons.

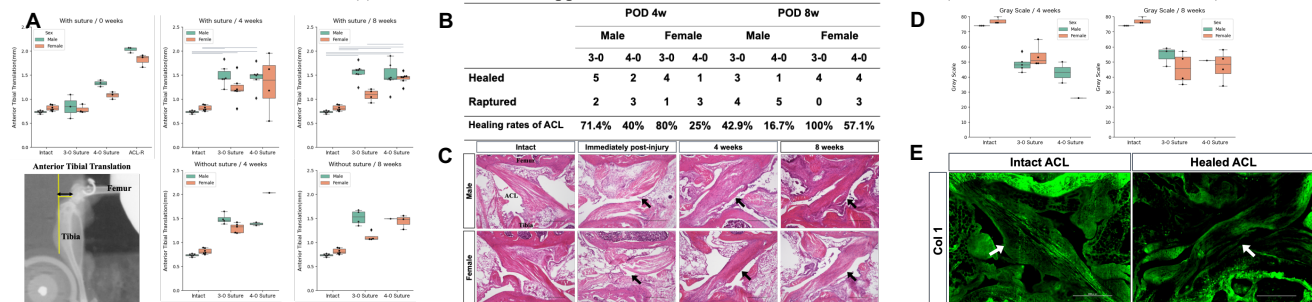
**RESULTS:** *Nylon Suture Experiments* In the failure testing, the load to failure was 1.4-fold higher for 3-0 nylon sutures. In cyclic testing, a 3-0 suture was 20 times stronger than a 4-0 suture in repeated stretching. *Anterior Drawer Testing (Fig. 1-A)* First, in the knees with CATT, immediately after injury, the knees with 3-0 sutures showed a trend toward smaller ATT compared with 4-0 nylon sutures ( $P = 0.20$ ). However, there was no significant difference in both Males and Females. At 4 and 8 weeks, ATT was significantly smaller in the Intact group in both Males and females except for females with 4-0 nylon sutures at 4 weeks after injury ( $P < 0.05$ ). Secondly, for the knees without nylon sutures, the ATT of the intact knee was smaller in both Males and females at 4 and 8 weeks. However, there were no significant differences because the number of samples counted as healed ACLs was small. *Histology* The 3-0 nylon sutures group had a high healing rate for both males and females (43~100%) (Fig.1-B). A higher grayscale calculated from PsR staining indicates higher ACL maturity. The grayscale of intact ACLs tended to be higher than that of healed ACLs (both of 3-0 and 4-0 nylon suture groups), but there were no significant differences among all groups. The localization of type I collagen in healed ACLs found no significant change in localization in all groups.

**DISCUSSION:** We investigated the effect of different braking forces of the ATT on the healing response of the completely injured ACL. As a result, we found that different braking forces significantly affected the success rate of ACL healing. The success rate of healing was low in the 4-0 group, which showed a higher ATT displacement, especially immediately after injury. The larger ATT probably pulled the ACL remnant apart and inhibited scar formation. Patients less than 20 years of age have been reported to have a lower success rate of conservative healing of ACLs due to their higher activity levels. In addition, excessive mechanical stress can cause degeneration of the healing tissue, even in tendon healing processes with surgical treatment. Thus, we showed that rigid braking of the ATT immediately after injury is important for the spontaneous healing response of the ACL. On the other hand, we observed healing in some ACLs in the 4-0 nylon suture group, which was loaded with higher mechanical stress. Healing of the ACL with a 4-0 nylon suture has the potential to be beneficial because mechanical stress on the ligament promotes extracellular matrix remodeling and increases the strength of the ligament. However, in the present study, there was no significant change in the production of type I collagen, and in the maturity of the ACL between 3-0 and 4-0 sutures. In conclusion, we showed that firm braking of the ATT, especially immediately after injury, is one of the factors that increase the success rate of healing during the spontaneous healing process of a completely injured ACL.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Our findings provide a basis for appropriate post-injury management and provision of orthotics and rehabilitation in ACL conservative treatment.

**REFERENCES:** [1] Menetrey+, *Injury*. 2011. [2] Filbay+, *BJSM*. 2023. [3] Kokubun+, *AJSM*. 2016. [4] Ihara+, *J Comput Assist Tomogr*. 2016.

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**Fig. 1.** (A) Results of Anterior Drawer Testing with and without nylon suture (B) Success rate of ACL healing (C) Representative of HE-stained images (D) Results of Gray Scale calculated from PsR-stained images (E) Representative images of IF-stained images