Comparison of Recovery Time Course for Spontaneous Healing vs Surgical Reconstruction in the Murine ACL Rupture Models

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INTRODUCTION: We reported that the ACL can heal spontaneously by controlling anterior tibial translation (CATT) after complete ACL injury [1]. A recent clinical report also showed that 90% of patients' ACLs healed with bracing protocol [2]. In our preliminary MRI-based verification, the patient showed that MRI signal intensity (SI) decreased as ACL healing progressed, indicating a trend toward maturation of the healed ACL (**Fig. 1A, B**). However, the time course of recovery compared to ACL reconstruction, which is the current gold standard treatment, remains unclear. While MRI data such as SI have recently been used to predict ligament and tendon healing [3], the actual ligament mechanical strength and biological mechanisms during the healing process cannot be elucidated. Therefore, in this study, we investigated the recovery in ACL healing and ACL reconstruction using the murine preclinical models.

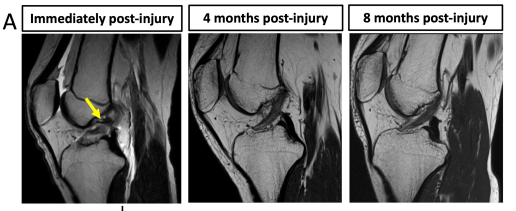

Fig. 1 (A) Representative MRI image of the ACL healing process. (B) Signal intensity time series data of the ACL.

METHODS: This study was approved by the Animal Research Committee of Saitama Prefectural University. We used 12-16-week-old male C57BL6 mice (n=24). First, we underwent a non-invasive ACL rupture in the left hindlimb under deep anesthesia. All mice were divided into the CATT group, which controlled the anterior displacement of the tibia to lead to spontaneous healing of the ACL, or the ACL-Reconstruction (ACL-R) group, which reconstructed the ACL using the tail tendon. Mice were sacrificed at 2 and 4 weeks after ACL injury. *Surgical Procedure*: In the CATT group, bone tunnels were created in the femoral condyle and tibia using a 26G needle after ACL injury. Then, a double 4-0 nylon suture was looped to stabilize the anterior displacement of the tibia (**Fig. 2A**). In the ACL-R group, 8–10 fibers of the tail tendon were harvested after ACL injury. Then, bone tunnels were created in the femur and tibia using a 27G needle. The tendon autograft was passed through the bone tunnel and fixed with a washer (**Fig. 2B**). *Anterior Drawer Testing*: (n=6-8/group and timepoint) Immediately after sacrificing the mice, anterior drawer testing was performed to evaluate knee instability in the CATT and ACL-R groups. *Cryohistology*: (n=3-4/group and timepoint) HE stains and immunofluorescence were performed. Immunofluorescence was used to assess the positive areas of type I collagen (Col1a1), and α-smooth muscle actin (α-SMA) in the healed and the reconstructed ACL. *Mechanical testing*: We performed failure testing to evaluate the mechanical strength of the healed ACL and the reconstructed ACL. *Statistics*: We analyzed for normality using the Shapiro-Wilk test. The two-way analysis of variance (ANOVA) was then performed to check for significant differences between the groups and the timepoints. Because all data were non-normally distributed, an Aligned Rank Transform was performed before ANOVA. Then, the Steel-Dwass test was performed as a multiple comparison.

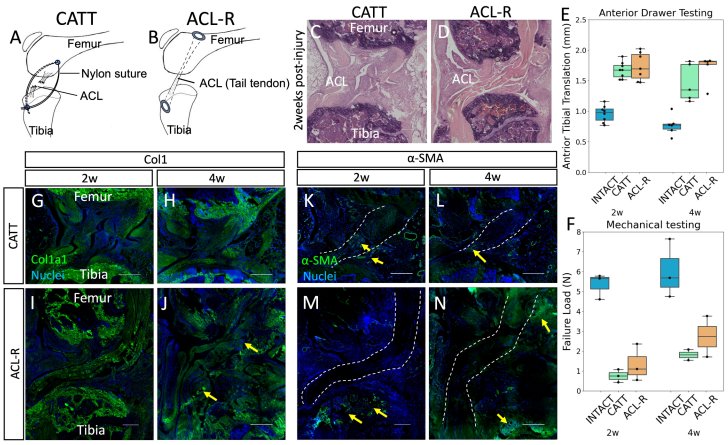

Fig. 2 (A) CATT and (B) ACL-R model. (C-D) HE-stained images at 2weeks. (E) Results of anterior drawer testing. (F) Results of mechanical testing. Representative images of col1a1 staining (G-J) and α-SMA (K-N). Scale bar = 100μm

RESULTS: *HE staining* We observed ACL reconnection in the CATT group and reconstructed ACL grafts in the ACL-R group at 2 weeks after injury (**Fig. 2C, D**). *Anterior Drawer Testing* Intact showed significantly lower anterior instability of the tibia than the CATT and ACL-R groups(p<.05). However, no significant differences existed between the CATT and ACL-R groups. Furthermore, CATT and ACL-R groups showed no significant differences between at 2 weeks and 4 weeks post-injury (**Fig. 2E**). *Mechanical testing*: Although the intact group showed significantly higher load to failure than the other groups (p<.05), no significant difference existed between CATT and ACL-R groups at 2- and 4-weeks post-injury (**Fig. 2F**). *Immunofluorescence* In the col1a1 staining, high expression of col1a1 was observe in the ACL remnants, and the mid-substance of the ACL appeared to have high-density nuclear aggregation at 2 weeks in the CATT group. At 4 weeks, col1a1 expression seemed to have spread throughout the ACL (**Fig. 2G, H**). In the ACL-R group, nuclear aggregation increased in the central region of the ACL at 4 weeks compared to 2 weeks. In the ACL-R group, few nuclei were observed in the high collagen expression area of the ACL at week 2, but at week 4, nuclear aggregation increased the interspaces in the collagen expression area (**Fig. 2I, J**). In α-SMA staining, α-SMA expression was observed in the ACL and intra-articular region in the CATT group (**Fig. 2E, F**). In the ACL-R group, α-SMA expression was not observed in the intra-articular region, but was observed in the bone marrow region near the ACL graft insertion site (**Fig. 2G, H**).

DISCUSSION: In this study, we compared the recovery of ACL spontaneous healing and ACL reconstruction with autograft tendons. As a result, we showed no difference in tibial instability and ACL mechanical strength between the CATT and ACL-R group. A clinical report suggested that patients with healed ACLs may have better recovery outcomes than those with reconstructed ACLs in 5 5-year follow-up. However, the short-term outcomes and the ACL function supporting them remain unclear. In animal model studies, healed ACLs showed 50-60% of normal mechanical strength by 4 weeks post-injury [1]. Reconstructed ACLs showed a significant decrease in mechanical strength at 1-week post-injury, and recovered to approximately 40% by 4 weeks, but showed no significant further changes [4]. This is the first study to compare healed and reconstructed ACLs, and the current results support these findings. We suggested that healed ACLs demonstrate a similar or greater level and time course of recovery compared to ACL reconstruction. Also, we revealed different healing mechanisms: ACL remodeling from within the joint capsule in ACL healing, and healing from outside the joint capsule at the tendon-bone attachment site in ACL reconstruction. It is known that ACL reconstruction involves host cell invasion and graft necrosis followed by remodeling, but the intra-articular healing mechanism in the ACL healing process is still unknown. Therefore, future research may reveal differences in proper rehabilitation by elucidating differences between the intra- and extra-articular healing processes, such as cell proliferation and matrix synthesis.

SIGNIFICANCE/CLINICAL RELEVANCE: We suggested that non-surgical treatment may lead to spontaneous healing of the ACL, potentially resulting in a similar or greater recovery than ACL reconstruction.

REFERENCES: [1] Kokubun+, ***AJSM.*** 2016. [2] Filbay+, ***BJSM.*** 2023. [3] Aitchson+, ***AJSM.*** 2021. [4] Yu+, ***J Orthop Translat.*** 2020.

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