

# Short-term Immobilization Followed by Treadmill Running Improves Tendon-to-Bone Healing in a Murine Model of Delayed Healing of Anterior Cruciate Ligament Reconstruction

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**INTRODUCTION:** Despite advances in surgical techniques, anterior cruciate ligament reconstruction (ACL-R) failure occurs in up to 12% of patients, often due to insufficient biological incorporation, resulting in graft failure and poor clinical outcomes [1]. ACL graft-to-bone healing requires bone ingrowth into the fibrovascular interface tissue that forms between the graft and bone tunnel, and data from our laboratory using animal models of ACL-R demonstrate that inferior graft load-to-failure is associated with poor bone ingrowth into the graft. We previously established a murine model of delayed healing of ACL-R by employing loose graft fixation in order to study the process of ACL graft incorporation (loose ACL-R model) [2]. As there is currently no consensus among clinicians regarding the use and duration of postoperative knee immobilization or weight-bearing following failed ACL-R, we therefore applied this model to evaluate whether postoperative rehabilitation strategies could enhance graft incorporation. We have previously demonstrated that both moderate treadmill exercise and short-term semirigid immobilization improve healing at the tendon-to-bone interface [3, 4]. The purpose of this study was to elucidate the effects of immobilization and treadmill exercise following loose ACL-R in mice. We hypothesized that short-term casting followed by treadmill loading would promote early tendon-to-bone healing and improve functional outcomes in this model of delayed healing following ACL-R.

**METHODS:** All experiments were approved by the Institutional Animal Care and Use Committee. Sixty 12-week-old male C57BL/6J mice (average weight: 26 g) were used in this study, and underwent open ACL transection followed by immediate loose ACL-R surgery in the right knee passing ipsilateral flexor digitorum longus tendon autograft in a tunnel with a 25% larger diameter than the graft with no pretension prior to graft fixation (loose fixation) and as previously described [2, 5]. The mice were randomly assigned to one of the four groups post-operatively: 1) free cage activity group, 2) 5 days cast immobilization followed by free cage activity (casting group), 3) treadmill running without casting (treadmill group), and 3) 5 days casting followed by treadmill running (casting + treadmill group) (Fig 1). All mice were euthanized at four weeks post-surgery. The primary outcome measures were biomechanical testing and measurement of new bone formation and tendon-to bone interface healing within the bone tunnel, assessed by micro-CT and histology (tendon-to-bone tunnel healing [TBTH] score [6]). Secondary outcomes included knee range of motion (ROM), and evaluation of anterior tibial translation (ATT) on a lateral view radiograph. Histological and biomechanical assessments were conducted on 5 and 10 mice per group, respectively, while the other measurements were conducted on 15 mice per group. Statistical analysis was carried out using one-way ANOVA followed by multiple comparisons using the Bonferroni test, with statistical significance set at  $p < 0.05$ .

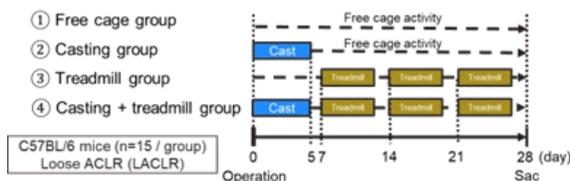
**RESULTS:** Though there were no significant difference in new bone formation and biomechanical ultimate load-to-failure, TBTH score in the femoral tunnel was higher in the casting groups compared to the free cage activity group (Fig 2). All groups exhibited restricted ROM compared to the contralateral knee. However, the casting group had significantly greater extension than the free cage group. The casting groups showed a tendency toward better extension angles compared with the non-casting groups, with the casting group demonstrating a significant improvement. In addition, the casting + treadmill group exhibited greater flexion angle and ROM than the treadmill group (Fig 3). Casting was associated with a reduction in ATT, especially when used in combination with treadmill loading.

**DISCUSSION:** Our findings indicate that short-term cast immobilization following loose ACL-R enhanced TB healing in the femoral tunnel and improved knee ROM compared with free cage activity. The casting group achieved significantly greater extension, while the casting + treadmill group showed superior flexion and overall ROM compared with treadmill exercise alone. We believe that the improvement in ROM despite a period of immobilization is due to resolution of the post-surgical inflammatory process. Subsequent controlled loading further facilitates ROM recovery. In addition, casting reduced ATT, particularly in combination with treadmill exercise, suggesting that short-term immobilization and controlled exercise may provide synergistic benefits. These results support the concept that early postoperative immobilization, followed by mechanical stimulation, may enhance TB healing and promote functional recovery in this model of delayed healing following ACL-R. Ongoing studies in our laboratory will quantify gait parameters as a further outcome measure of pain and function. Importantly, these observations extend our previous reports on semi-rigid casting and treadmill running by demonstrating their combined benefit in a delayed healing ACL-R model. Although modest improvements were observed, the results highlight the potential of targeted rehabilitation strategies to improve outcomes after ACL-R. Further studies with longer follow-up are warranted to validate these effects and explore underlying mechanisms.

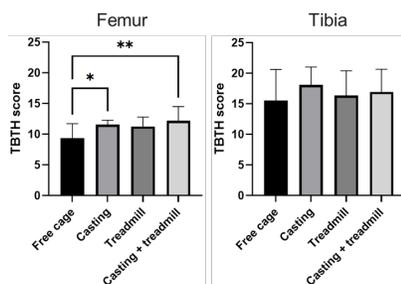
**SIGNIFICANCE/CLINICAL RELEVANCE:** This study demonstrates that short-term casting followed by treadmill exercise improves TB healing, knee ROM, and anterior stability in a murine model of delayed ACL-R healing. These findings suggest that an initial period of immobilization followed by controlled loading may represent a clinically relevant rehabilitation strategy to enhance biological incorporation and functional recovery after ACL-R.

**REFERENCES:** [1] Crawford SN et al. Arthroscopy 2013. [2] Morita W et al. ORS 2024. [3] Okazaki Y et al. ORS 2024. [4] Suzuki Y et al. ORS 2025. [5] Lebaschi A et al. J Knee Surg. 2017. [6] Liu PP et al. Anal Quant Cytol Histol 2011.

**Figure 1:** Scheme of the postoperative plans of each group. TM: treadmill.



**Figure 2:** Histological evaluation: tendon-to-bone healing (TBTH) scores. \* $P < 0.05$ , \*\* $P < 0.01$ .



**Figure 3:** Secondary outcomes: (A) knee range of motion (ROM), extension angle, and flexion angle. (B) radiographic evaluation of anterior tibial translation (ATT). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

