

# Graft Press-Fit Promotes Tunnel Integration After ACL Reconstruction

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**INTRODUCTION:** The anterior cruciate ligament (ACL) is frequently injured, resulting in over 200,000 reconstruction procedures performed annually in the U.S. alone. The ACL reconstruction (ACLR) procedure involves drilling bone tunnels that are filled with tendon grafts to replace the native ACL. Lineage tracing studies demonstrate that the cells within the tendon graft do not actively contribute to the tunnel integration process. Instead, progenitor cells from the bone marrow outside the tendon graft activate in response to the tunnel injury, proliferate, and then migrate into the tendon graft to form mineralized fibrocartilage (MFC)-containing zonal tendon-to-bone attachment sites during the tunnel integration process [1-3]. Successful integration of the tendon graft with bone is critical to reach pre-surgical functional levels. Unfortunately, a common complication of ACLR is tunnel widening, which can be caused by improper tunnel positioning, excessive motion, or fluid effusion into the tunnels, often leading to synovial cysts in the tunnels [4]. Proper sizing of the tendon graft to the tunnel (i.e., press-fit) to minimize motion and fluid effusion is thought to be important for promoting tendon-to-bone integration while minimizing tunnel enlargement and, ultimately, graft failure [5]. Therefore, the objective of this study is to determine the extent to which graft fit affects tendon-to-bone integration following ACLR. Our hypothesis is that a properly fit tunnel will promote integration.

**METHODS:** All animals and procedures were approved by our institution's IACUC. **Experimental Design:** ACLR was performed on 43 mice, which were split into three groups. In the control group, the bone tunnels were drilled with a 27G needle and received our standard 1 mm diameter graft shown to produce zonal attachments [1-3]. We then modified the press-fit by either increasing the size of the tunnel with the same size graft (25G) or by decreasing the size of the graft with the same size tunnel (27G Half) (**Fig 1A**). Mice from all groups were assessed at 28 days post-surgery. **ACL Reconstruction:** The ACL was surgically transected. Tibial and femoral tunnels were drilled with 25G or 27G needles. Tail tendon autografts (~1 mm and ~0.5mm diameters for 1X and 1/2X grafts, respectively) were passed through the tunnels and anchored with external fixators. A 100-gram-force dynamometer dial gauge was used to measure the force required to pull the graft through the tunnel (n=9-12/group). **Mineralized Cryohistology:** Knees (n=13-15/group) were fixed in formalin, embedded, sectioned undecalcified with cryofilm, and imaged using a Zeiss Axio Scan.Z1. **Image Quantification:** MFC area, cyst area, tunnel length, and tunnel area were measured with ImageJ. Cyst area was defined as void spaces with curved edges in-between graft fibers, including cystic and mucoid degeneration. Average tunnel width was calculated by dividing the tunnel area by its length. **Statistics:** Tunnel dimensions and MFC area were compared between groups via one-way ANOVAs with Tukey's posthoc. Dynamometer readings and cyst area were compared with Kruskal-Wallis tests with Dunn's posthoc.

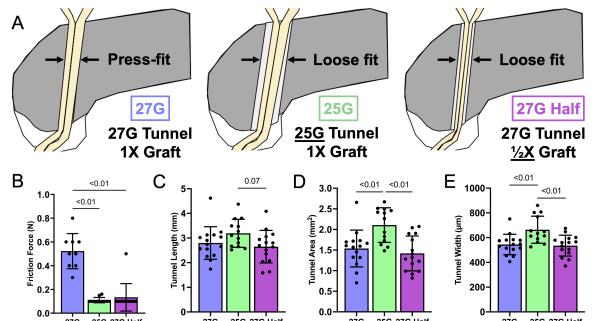
**RESULTS:** The force required to pull the graft through the tunnel during surgery was higher in the 27G group compared to the 25G and 27G Half groups (p<0.01, **Fig. 1B**). At 28 days post-surgery, the 25G group had higher tunnel area and tunnel width compared to the 27G and 27G Half groups (p<0.01, **Fig. 1C-E**), with tunnel widths aligning with the diameters of a 25G or 27G needle. The 27G group promoted MFC formation compared to the 25G (p<0.01) and 27G Half (p=0.01) groups (**Fig. 2B**). Conversely, the 25G group had greater cyst area than the 27G group (p=0.04) but not the 27G Half group (**Fig. 2D**). MFC area did not correlate strongly with cyst area, with the 27G group having higher MFC and the other groups having higher Cyst values (**Fig. 2E**). Interestingly, MFC area positively correlated with tunnel length in the 27G group (Pearson coefficient 0.64, p<0.01, **Fig. 2F**), and cyst area correlated with tunnel width in the 25G group (Pearson coefficient 0.62, p=0.01, **Fig. 2G**). Finally, we investigated the position of cysts in the tunnel and found that cysts were generally closer to the joint space and that the 25G group had a higher percentage of cysts extending deeper into the tunnel, possibly from the loose fit and larger tunnel size (**Fig. 2H**).

**DISCUSSION:** During the early stages of healing post-ACLR, graft fixation plays a stabilizing role. Our histological analysis revealed greater tendon-to-bone integration (as indicated by more MFC), and less cyst formation in the tighter-fitting 27G group. In fact, hardware-free press-fit ACLR procedures, in which the graft fits tightly in the tunnels, are being investigated as techniques to reduce fluid effusion and tunnel enlargement compared to traditional ACLR procedures with fixation hardware [4,5]. As expected, the tunnels were wider in the 25G group in our study and may have contributed to higher incidence of cysts that were deeper in the tunnels. In addition, we found that tunnel length positively correlated with MFC area in tighter fitting 27G tunnels and tunnel width correlated with cyst area in the looser 25G tunnels. The looser-fitting tunnels generally had more remodeling of the tendon graft than the press-fit, potentially resulting in more opportunities for cysts formation. However, due to the nature of the tail tendon graft having multiple fascicles, the tension in each fascicle is likely different regardless of the fit of the graft in the tunnel, possibly explaining why cysts occurred in press-fit tunnels as well.

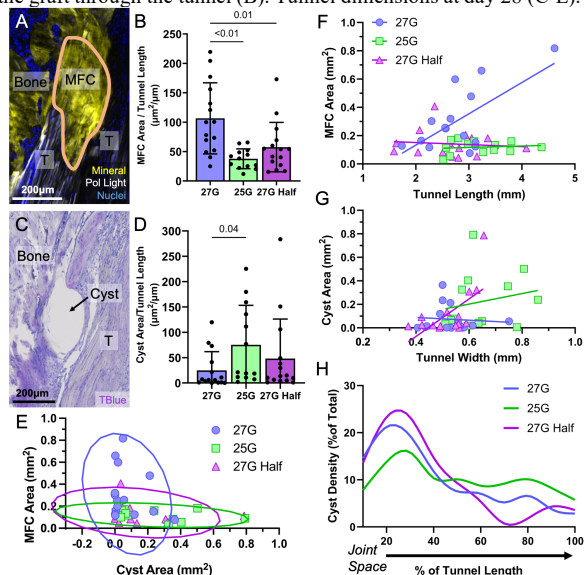
**SIGNIFICANCE/CLINICAL RELEVANCE:** This study emphasizes the importance of properly fitting the graft to the bone tunnel in ACLR, informing surgical practice. Additionally, this murine surgical model can elucidate specific cellular mechanisms related to press fit in the future using transgenic mice.

**REFERENCES:** 1. Kamalitinov et al., *JOR*, 2020; 2. Hagiwara et al., *Ann NY Acad Sci*, 2020; 3. Kamalitinov et al., *Osteoarthritis Cartil*, 2023; 4. Rizer et al., *Skeletal Radiol*, 2017; 5. Paessler and Mastrokalos, *Orthop Clin North Am*, 2003.

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**Fig. 1:** Tunnel diameter and graft size were used to modulate the press-fit of the graft (A). Press-fit tunnels required more force to pull the graft through the tunnel (B). Tunnel dimensions at day 28 (C-E).



**Fig. 2:** Press-fit promoted MFC (A-B) and reduced cyst formation (C-D). MFC and cyst formation did not correlate (E). MFC (F) and Cyst (G) relationships to tunnel dimensions. Location of cysts as a function of distance from the joint space (H). T = tendon graft.