Elucidation of the Meniscus–Bone Healing Process investigated with Pull-Out Repair Model for Medial Meniscus Posterior Root Tear in Rats

Toyohiro Katsumata[1], Yusuke Nakagawa[1], Masaki Amemiya[1], Mai Katakura[1], Ryohei Takada[1], Tomomasa Nakamura[1], Kunikazu Tsuji[1], Ichiro Sekiya[2], Hideyuki Koga[1].

1. Department of Joint Surgery and Sports Medicine, Institute of Science Tokyo, Tokyo, Japan.
2. Department of Applied Regenerative Medicine, Institute of Science Tokyo, Tokyo, Japan.

katsumata.orj@tmd.ac.jp

Disclosures: Authors have nothing to disclose.

INTRODUCTION: Pull-out repair for medial meniscus posterior root tear (MMPRT) is widely adopted, but the time course and modes of meniscus–bone healing after this technique remain poorly characterized. Prior animal studies are limited, and the repair-site healing process is not well understood [1,2]. We aimed to establish a rat model of MMPRT pull-out repair incorporating postoperative immobilization. In this study, the model quantifies medial meniscal extrusion (MME) using micro-CT, measures repair-site tensile strength via biomechanical testing, and delineates meniscus–bone healing histologically. We hypothesized that pull-out repair, with the meniscal stump seated in a tibial bone tunnel, would induce ACL reconstruction–like indirect (fibrovascular) healing at the meniscus–bone interface, thereby suppressing MME and regaining biomechanical properties.

METHODS: All animal experiments were approved by our Institutional Animal Care and Use Committee. One hundred nine-week-old male Wistar rats were randomized to Repair group (R group) or Non-R group (Non-R group); contralateral knees served as internal controls for micro-CT–based MME and biomechanical testing. **Surgical procedure:** All procedures were performed on right knees under general anesthesia. In both groups, after incising the posteromedial capsule via medial approach, we created a LaPrade type 2 MMPRT [3] (Fig.2A). In the R group, a Delta-grip stitch [4] was applied to the meniscal stump (Fig.2B), and a 1.0-mm tibial tunnel was drilled from an articular starting point medial to the PCL footprint to an anterolateral exit (Fig.2C, D). Sutures were routed and secured with a cortical suture button (Fig.2E, F), seating the meniscal stump within the bone tunnel. In both groups, rats were immobilized in knee extension for 2 weeks using an external fixator (Fig.2G, H). Animals were euthanized at 4 or 12 weeks for examinations. **Micro-CT**: Meniscus extrusion ratio (MER) was measured using PTA-enhanced micro-CT imaging, referencing a previously reported method for the imaging technique [5]. (n = 5 per group per time point; 20 contralateral controls). MER was defined as b/a on coronal images at the meniscal body level, where 'a' is the total medial meniscus length and 'b' is the extruded length [6] (Fig.3A). **Biomechanical testing**: Maximum load to failure was measured using axis-aligned distraction along the bone tunnel (n = 12 per group per time point); contralateral samples were included in a blinded manner (n = 4 per group per time point; total n = 16). **Histology:** Paraffin sections in both groups were stained with hematoxylin and eosin (H&E) and Safranin O. Sections from the R group were cut along the bone-tunnel axis to assess meniscus–bone interface healing, whereas sections from the Non-R group were prepared in sagittal or coronal planes to evaluate post-tear fibrous healing. Statistics used one-way ANOVA with Tukey's post hoc; P values < 0.05 were considered statistically significant. Values are shown as mean ± SD (range).

RESULTS: **Micro-CT (MER)**: The R group did not differ significantly from contralateral knees at 4 or 12 weeks (healthy, 0.16 ± 0.09; Repair-4w, 0.18 ± 0.13; Repair-12w, 0.22 ± 0.04). The Non-R group showed significantly greater extrusion than both the R group and healthy controls at each time point (Non-R-4w, 0.55 ± 0.18; Non-R-12w, 0.50 ± 0.07; all $p < 0.001$) (Fig.4). **Biomechanical testing**: Maximum load to failure (N) was 10.3 ± 3.68 (healthy), 7.56 ± 2.96 (R-4w), 8.84 ± 3.71 (R-12w), 3.53 ± 1.69 (Non-R-4w), and 7.33 ± 2.77 (Non-R-12w). At 4 weeks, the Non-R group exhibited significantly lower than the R group at both 4 and 12 weeks and healthy controls ($P < .05$, $P < .01$, or $P < .001$). By 12 weeks, he Non-R group did not differ significantly from the R group or healthy controls (Fig.5). **Histological evaluation**: In the R group, 7/8 specimens at each time point retained the meniscal stump within the tunnel. At 4 weeks, a fibrovascular interface consistent with indirect healing bridged the meniscus–tunnel wall, with disorganized collagen and evident angiogenesis (Fig.7). By 12 weeks, fiber alignment improved but remained irregular (Fig.8); two specimens showed direct-type attachment near the intra-articular tunnel aperture (Fig.8). In the Non-R group, no reattachment to the native footprint was seen at 4 weeks; by 12 weeks, proliferative fibrous tissue adhered to the PCL, original footprint, and adjacent articular cartilage (Fig.6).

DISCUSSION: Our primary finding was that meniscus-to-bone healing following pull-out repair proceeded predominantly via an indirect fibrovascular interface, with some degree of focal direct-type healing. A secondary finding was that the pull-out repair suppressed MME to levels approaching those of intact knees and produced a tensile strength at 12 weeks that was comparable to the contralateral knee. The Non-R group showed substantial tensile strength at 12 weeks. However, the loss of hoop function due to persistent MME demonstrates that the tensile properties of the scar tissue do not equate to the functional restoration of the meniscus.

SIGNIFICANCE/CLINICAL RELEVANCE: Clarifying meniscus-bone healing mechanism may guide the development of future treatment strategies for human MMPRT pull-out repair, informing choices regarding fixation and augmentation strategies to promote interface maturation and protect the repair.

REFERENCES: [1] Deng XH et al. Arthroscopy 2024. [2] Dzidzishvili L et al. Am J Sports Med 2023. [3] LaPrade CM et al. Am J Sports Med 2015. [4] Ishibashi et al. Arthroscopy Techniques 2024. [5] Lesciotto KM et al. Dev Dyn 2020. [6] Ozeki N et al. J Orthop Sci 2017.

Fig.1

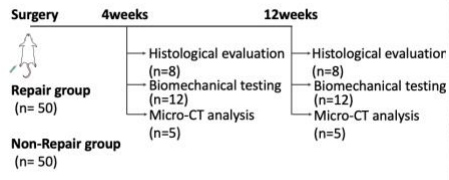
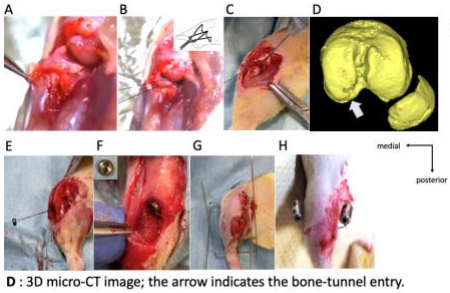


Fig.2



D : 3D micro-CT image; the arrow indicates the bone-tunnel entry.

Fig.3

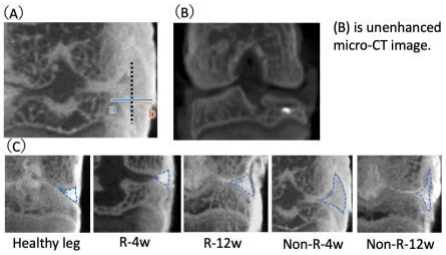


Fig.4

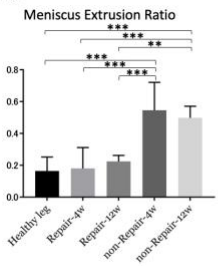
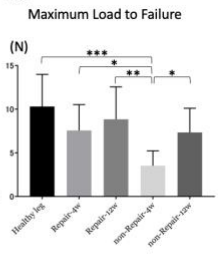


Fig.5



* P < .05; ** P < .01; *** P < .001

Fig.6

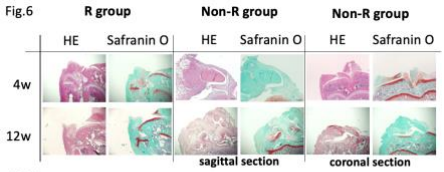


Fig.7

Fig.8