Early joint immobilization induced changes in self-healing ligament in anterior cruciate ligament injury

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INTRODUCTION: The anterior cruciate ligament (ACL) of the knee was traditionally considered non-healing; however, reports suggest that self-healing is achievable by restraining abnormal joint motion after injury. Nonetheless, a challenge remains for clinical implementation, as the healing ACL (CAM) is observed to be more fragile compared to the intact ligament (INTACT) (Figure 1). Our previous research demonstrated that tension stress changes induced in the ACL during joint motion and stretching stress on ligament-derived fibroblasts enhance the expression of ligament-related genes (ORS Annual meeting, 2023), and we also revealed that joint immobilization (IMM) over 8 weeks following ACL self-healing surgery leads to a decrease in strength (Figure 1). Consequently, we indicated the potential for stretching stress to reinforce ligament resilience and highlighted the necessity of stress application during appropriate healing periods. The subsequent challenge is to verify whether modulation of stretching stress according to the healing process affects ligament mechanical strength. For this reason, in this study, we aim to utilize a model that reduces stretching stress on early post-rupture healing ACLs to elucidate the impact of early post-rupture stretching stress on ligament strength.

METHODS: This study was approved by the Ethics Committee of Saitama Prefectural University and strictly adhered to the on-campus animal experiment guidelines (approval number: 2022-4).In this study, eighteen male Wistar rats (aged 10 weeks, 240±10g) were used. All rats underwent surgical tearing of the right knee ACL under anesthesia and underwent arthrodesis to enhance self-healing according to previous studies. This model only restricts abnormal joint movement, and the knee joint range of motion is maintained. After surgery, the animals were divided into two groups—a normal group (CAM: Control Abnormal Motion)(n=9) and a 2weeks joint immobilization group (2wIMM: 2weeks Immobilization)(n=9). In the 2wIMM group, the knee joint was externally immobilized in a flexed position of 90 degrees immediately after surgery for a period of two weeks. External immobilization was achieved by placing pins in the femur and tibia, connecting these pins with wires. The immobilization was immediately released by cutting the wires when removing the fixation.At the end of the experimental period (8 weeks), all rats were sacrificed. The right knee joints of three rats in each group were subjected to histological analysis; for the other samples, all soft tissues, except the ACL, were removed, and the maximum rupture strength and the tissue tensile distance to rupture were measured using the UnivVert tensile strength testing machine (Orange Science, JPN). The results of the mechanics test were compared between the CAM and 2wIMM groups using an uncorrelated t-test. The significance level was set at <0.05 for all analyses.

RESULTS SECTION: Histologically, all the ACLs in the CAM and 2wIMM groups had healed (Figure 2). In the tensile strength test, the maximum breaking strength averaged 13.07 N (95% CI: 10.95-15.19 N) in the 2wIMM group and 8.88 N (95% CI: 5.91–11.85 N) in the CAM group; thus, it was significantly higher in the 2wIMM group (p=0.003). Tensile distance averaged 0.84mm (95% CI: 0.57-1.11 mm) in the 2wIMM group and 0.99mm (95% CI: 0.58-1.40 mm) in the CAM group. No significant difference was seen between CAM group and 2wIMM group (p=0.170)(Figure 3).

DISCUSSION: An interesting finding of this study is the increased ligament strength resulting from early joint immobilization following the healing intervention. Tissue healing is generally categorized into three phases: inflammation, proliferation, and remodeling. The initial inflammatory phase responds poorly to mechanical stimuli, whereas the later remodeling phase is receptive to mechanical stimuli, promoting healing (Eliasson P, et al.). The two weeks post-surgery in this study correspond to the inflammatory and proliferative phases, during which joint immobilization may have prevented prolonged inflammation and microscopic tissue damage, potentially facilitating healing. In essence, stretching stress is likely more relevant to the remodeling phase than the inflammatory and proliferative phases. We anticipate that further investigations in this direction can offer novel foundational insights into the healing process and rehabilitation following complete rupture.

SIGNIFICANCE/CLINICAL RELEVANCE: This study contributes to the enhancement of strength in the clinical application of self-repairing ACLs.

IMAGES AND TABLES:

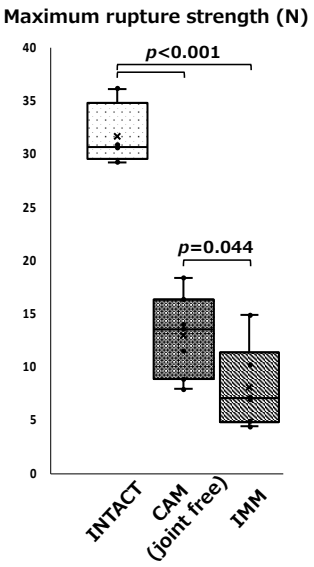

Figure 1

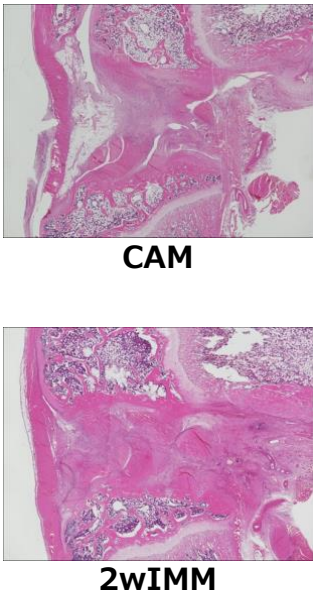

Figure 2

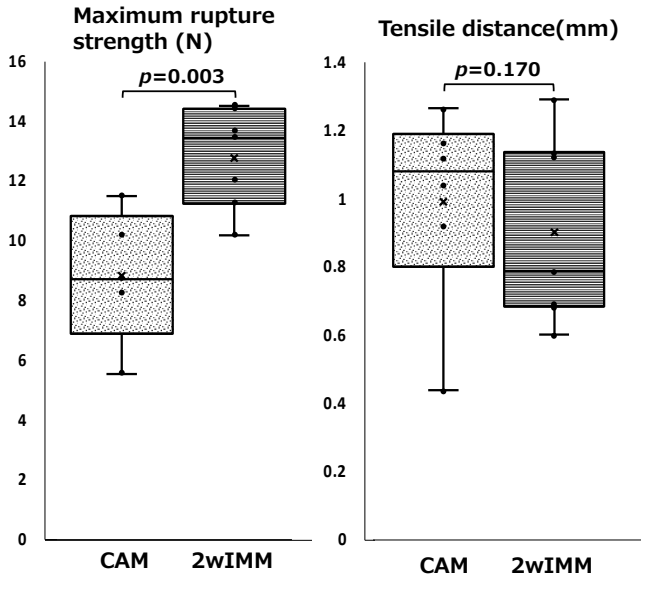

Figure 3