Repair potential of self-assembling peptide hydrogel scaffold for anterior cruciate ligament reconstruction in a mouse model

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INTRODUCTION: Successful anterior cruciate ligament reconstruction (ACLR) hinges on the establishment of a strong zonal attachment between the graft tendon and the bone tunnel. However, the healing process is typically slow, resulting in disorganized attachment and poor mechanical properties compared to the native ACL. Self-assembling peptide hydrogels (SAPS) have been used as scaffolds to improve the healing process in various fields. We developed KI24RGDS (IKIKIKIKIKRGDSKIKIKIKIKI, where K = lysine, I = isoleucine, R = arginine, G = glycine, D = aspartic acid, and S = serine), a SAPS with the RGDS amino acid sequence. Ligands have been incorporated into biomaterials to promote cell proliferation and interactions [1]. In this study, we elucidate the therapeutic potential of KI24RGDS in facilitating zonal tendon-to-bone attachment after ACLR in a Maurine ACLR model. We hypothesized that KI24RGDS contributes as a scaffold between the graft tendon and the bone tunnel, advancing zonal establishment by promoting progenitor cell proliferation.

METHODS: All procedures were approved by the Institutional Review Board of the authors' university. Sixty-two male C57BL/6 mice (aged 12-13weeks) were divided into the ACLR + SAPS group (n = 40, including 16 FITC labeled KI24RGDS (SAPS + FITC)) and the ACLR group (n = 24). ACLR were performed using the tail tendon according to the protocol described previously [2]. In the ACLR+SAPS Group, KI24RGDS was attached to the tail tendon before tendon fixation. Mineralized Cryohistology. To assess the maturation of the tendon-to-bone attachment, we quantified the area of mineral fibrocartilage (MFC) in the graft tendon at days 7, 14, and 28 post-ACLR (n = 4/group). One day before euthanasia, the mice were intraperitoneally injected with demeclocycline. Sagittal sections (6 μm thick) were produced using a cryostat and mounted according to Kawamoto's methods [3].KI24RGDS Localization and Immunohistology. To evaluate the area of KI24RGDS attached to the graft tendon and the change in the area after ACLR at days 0, 7, 14, and 28, the ACLR + SAPS group (n = 4, SAPS + FITC) was observed under a fluorescence microscope. Subsequently, immunofluorescence staining for α smooth muscle actin (αSMA) was performed (n = 4/group). Tunnel Pull-out Test. To evaluate tendon-to-bone attachment strength, a pull-out test was performed on days 14 and 28 after ACLR (n = 6/group). The right femur was dissected and the connective tissue around the femur was removed; however, the washer and graft tendon were retained. A 2-0 nylon suture was passed through the washer and the suture end and proximal femur were placed in plastic pots and fixed with polycaprolactone. The suture was pulled parallel to the bone-tunnel axis (10 mm/min) until the graft tendon was removed. Image Quantification. ImageJ was used to measure the mineralized fibrocartilage area (MFC), tunnel length, SAPS + FITC area, the number of α SMA positive cells, and total cell count.Statistics. One-way ANOVA was used to compare FITC areas between groups, while two-way ANOVA ($p < 0.05$) was employed for pullout strength and histomorphometric parameter comparisons.

RESULTS: Mineralized Cryohistology: At day 14, the ratio of MFC to tunnel length was significantly increased compared to that on day 7 in both groups (Fig.1). Notably, MFC within the ACLR + SAPS groups was markedly higher than that in the ACLR group at day 14. However, no significant disparity in the ratio was found between the two groups on day 28.KI24RGDS localization and immunohistology: KI24RGDS in the bone tunnel was sufficiently attached to the graft tendon and occupied the space between the graft tendon and the bone tunnel on day 0 after ACLR (ratio to tunnel area was 68.5%). The area of KI24RGDS decreased significantly by day 28 after ACLR compared to day 0. However, 44.3% of KI24GDS remained within the tunnel. The number of α SMA positive cells in graft tendon was the highest at day 7 after ACLR in both groups (Fig.2). On day 14, the numbers were markedly reduced compared to those on day 7. On day 30, few α SMA-positive cells were observed. On day 7, the number was significantly higher in the ACLR + SAPS group than in the ACLR group. Tunnel Pull-out Test. Biomechanical analysis indicated that the maximum failure load in the ACLR + SAPS group was significantly higher than that in the ACLR group at day 14 and 28 after ACLR. Importantly, both groups displayed significantly greater maximum failure loads at day 28 than at day 14. However, no significant differences were observed between the two groups at day 28. (Fig.3)

DISCUSSION: SAPS, composed of peptide nanofibers that form a 3D structure, have a with pore diameter similar to natural extracellular matrix components. These characteristics promote cell proliferation and interaction without cytotoxicity and immune response. Our findings underscore the supportive role of KI24GDS in promoting the proliferation of αSMA-positive cells within the bone tunnel. Acting as a scaffold between graft tendon-to-bone tunnel, KI24GDS facilitates early-stage tendon-to-bone attachment formation. As the success of the rehabilitation protocol after ACLR pivots on the establishment of secure attachment between the graft tendon and bone tunnel, the use of the KI24GDS may accelerate the rehabilitation process and shorten the return to sporting activities. However, the ACLR + SAPS group at day 28 post-ACLR demonstrated that KI24GDS did not change the MFC and mechanical properties of tendon-to-bone attachment. The focus solely on supporting progenitor cell proliferation as a scaffold did not improve the zonal attachment quality. While the precise healing mechanism of the enthesis remains unclear, a previous study suggested that a combination of tissue engineering and bioaugmentation may enhance the attachment of mature zonal tendons. Further studies are needed to clarify the impact of KI24GDS bioaugmentation on ACLR.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights the latent healing potential of KI24GDS in facilitating early-stage zonal attachment in graft tendons and bone tunnels post-ACLR. These findings may expediate the rehabilitation protocols and the timeline of returning to sporting activities.

REFERENCES: 1. Hirano et el., J Biomed Mater Res. 1991; 2. Kamalitdinov et al., JOR, 2019; 3. Kawamoto et el., Methods in Mol. Biol. 2021

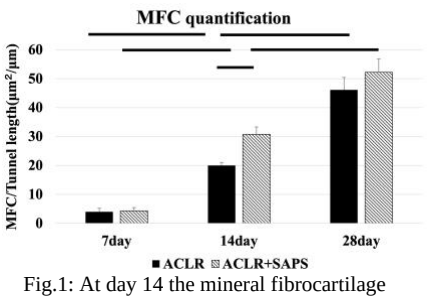

Fig.1: At day 14 the mineral fibrocartilage area (MFC) in the ACLR + SAPS group was significantly greater than in the ACLR group. Bar denote $p < 0.05$

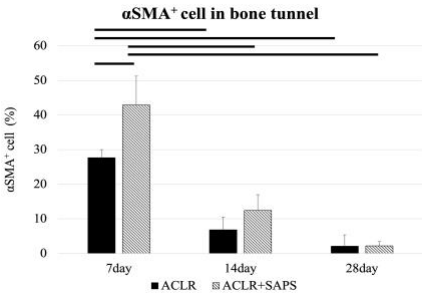

Fig.2: The percentage of cells in the ACLR + SAPS group was significantly higher than the ACLR group at day 7.

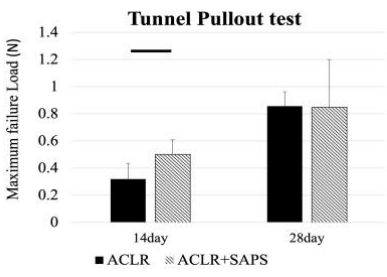

Fig.3: Maximum failure load in the ACLR + SAPS group was higher than in the ACLR group at day 14. Bar denote $p < 0.05$