A Murine Model of Anterior Cruciate Ligament Reconstruction Failure

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INTRODUCTION: A myriad of studies have investigated strategies to improve healing after anterior cruciate ligament reconstruction (ACL-R) surgery, utilizing animal models for pre-clinical research to identify the factors that contribute to ACL-R healing. However, to date there is no model that models failure of ACL-R, graft loosening and consequent post-traumatic osteoarthritis (PTOA). ACL-R failure may occur in up to 12% of the operated patients and be attributed to insufficient biological incorporation leading to graft failure and inferior clinical outcomes.[1]. The purpose of this study was to create a murine model of failed ACL-R healing. We hypothesized that loose fixation of the graft would result in failed ACL-R healing that is analogous to clinical ACL-R failure, and that comparison to the conventional ACL-R model (standard) would identify key mechanisms related to failed ACL-R healing that can be therapeutically targeted.

METHODS: All experiments were approved by the institutional animal care and use committee. We have previously developed a mouse model of ACL-R using the ipsilateral flexor digitorum longus tendon as the graft.[2] We designed a new model with a loose fixation of the graft by surgical approach of creating a tunnel with a 25% larger diameter and applying no pretension prior to graft fixation (loose fixation), which were identified as perioperative factors that affect ACL-R healing amongst post-op loading protocols through a systematic review of the literature. A total of 90 twelve-week-old male C57BL/6 mice underwent either standard or loose fixation ACL-R surgery and were allowed to weight-bear immediately after recovery from anesthesia post-op. Mechanical testing of graft strength was measured by ultimate load to failure (n = 9 per group). ACL-R healing and cellular composition was assessed by histology and measuring bone formation within the bone tunnel by micro-CT (n = 11 per group). Stromal cell populations were determined using *Bglap*.eGFP reporter mice, which identifies stromal cells that can contribute to bone formation activity in the bone tunnel (n = 3 per group), and flow cytometric analysis (n = 12 per group). PTOA development was assessed by plain radiographs using the Kellgren-Lawrence grading scale and histology (n = 10 per group). Gait analysis was used as a measure analogous to clinical outcomes (n = 10 per group). Non-parametric tests were used to compare the differences between the two models. Statistical significance was set at $p < 0.05$. Data shown as median with interquartile range and p values are indicated in the figures.

RESULTS: We have created a murine model of ACL-R failure characterized by fibrotic bone-to-tendon healing and decreased bone formation within the bone tunnel resulting in impaired biomechanical properties of the graft complex. Ultimate load to failure was significantly decreased in the loose fixation model compared to the standard model at 4 weeks post-op (Figure 1). However, gait was not affected at 4 weeks post-op (Figure 1). Hematoxylin and eosin staining showed delayed and incomplete ACL-R healing with inferior bone-to-tendon integration and development of fibrotic scar tissue at the interface in the loose fixation model from 2 weeks post-op (Figure 2). New bone formation within the bone tunnel increased until 4 weeks post-op and plateaued at 8 weeks post-op in the standard model. This was significantly decreased in the loose fixation model at 4 weeks post-op (Figure 2). Immunofluorescence showed diminished *Bglap* positive bone-forming cells in the loose fixation model relative to the standard model (Figure 3). Consistently, interface tissue cells in the loose fixation model showed distinct differences in stromal progenitor cells (CD45- TER119- CD31- THY1.2- 6C3+) compared to the standard model, suggesting their dysregulated recruitment in inferior ACL-R healing. Intriguingly, PTOA development was accelerated in the loose fixation model compared to the standard model at 4 weeks post-op (Figure 3).

DISCUSSION: The results of this study depict the inferior healing of ACL-R causing mild, accelerated, and subclinical OA development at 4 weeks post-op. Our data also suggest that the loose fixation of the graft results in fibrotic healing at the bone tunnel interface by recruiting preferentially a subset of stromal cells including fibroblast precursors. Further mechanistic studies are necessary to elucidate the pathobiology of fibrotic healing and signals for the increased stromal progenitor cells.

SIGNIFICANCE/CLINICAL RELEVANCE: This murine ACL-R failure model is the first to our knowledge clinically relevant model of PTOA after ACL-R. This ACL-R loose fixation model enables mechanistic studies of failed ACL-R and identification of therapeutic interventions to prevent fibrotic bone-to-tendon healing and graft failure.

REFERENCES:

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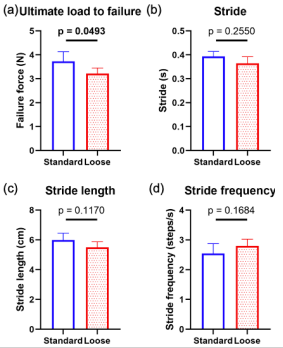

Figure 1. (a) Mechanical strength of the graft complex assessed by ultimate load to failure at 4 weeks post-op. (b - d) Gait analysis at 4 weeks post-op.

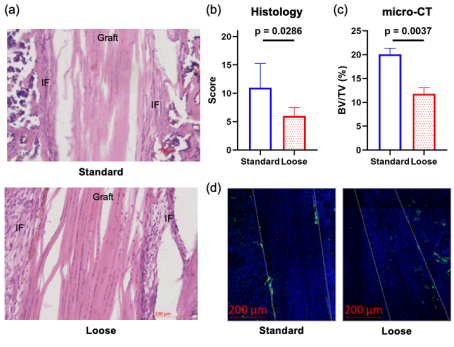

Figure 2. (a) Representative histology images and (b) scoring at 2 weeks post-op. IF, interface. (c) Bone formation measured by bone volume fraction (BV/TV) at 4 weeks post-op. (d) Representative immunofluorescence images of *Bglap*.eGFP (green) at 4 weeks post-op. Bone tunnel shown by dashed lines.

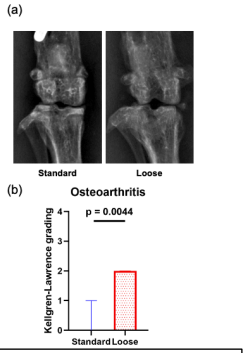

Figure 3. (a) Representative anterior to posterior plain radiographs and (b) grading of OA development at 4 weeks post-op.