From Heart to Shoulder: In Vitro and Ex Vivo Therapeutic Evaluation of Recombinant Periostin in the Rotator Cuff Overuse Tendinopathy Model via Novel Cell and Tendon Strex Bioreactors

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INTRODUCTION: Rotator cuff tendinopathy (RCT) is a prevalent overuse disorder characterized by extracellular matrix disorganization and poor healing. Despite advances in rehabilitation and surgery, biological solutions that restore tendon resilience remain elusive. In contrast, cardiac chordae tendineae (CT) experience billions of loading cycles without rupture, suggesting intrinsic protective mechanisms. Transcriptomic profiling identified Periostin (POSTN) as one of the most enriched CT matrix proteins. We hypothesized that POSTN enhances tendon stem cells' (TSC) survival and tenogenesis under mechanical overload, thereby conferring resilience against tendinopathy.

METHODS: Healthy mongrel dogs (n = 3, 10–15 months, 20–25 kg) provided CT and limb tendons (LT) for RNA sequencing. Only male dogs were selected to reduce hormonal and cyclic differences that could confound gene expression comparisons between different tendon types in small-sample RNA-seq analyses. Healthy or Grade 3 hypertension domestic pigs (n = 38, 3–4 months, 40–65 kg, mixed sex, female: male = 1:1) provided CT TSC, CT strips, infraspinatus (IST) TSC, and IST strips for both in vitro and ex vivo experiments. All procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). Bulk RNA-seq (Illumina HiSeq 4000) compared CT and LT transcriptomes (FDR < 0.05, $|\log_2 FC| > 2$). Two programmable bioreactors—Cell Strex (in vitro) and Tendon Strex (ex vivo)—applied cyclic uniaxial strain (0–9%, 0.25–0.5 Hz) to mimic physiological and overload tendinopathic conditions. Recombinant human POSTN (1 µg/mL) was added during stimulation in the treatment group. Cell proliferation, apoptosis, and migration were quantified by IncuCyte imaging; collagen morphology by SEM/TEM; and tenogenic gene expression (Col I a1, Scx, Mkx, Tnmd) by RT-qPCR ($p < 0.05$).

RESULTS SECTION: Bulk RNA-seq of three healthy male canine chordae tendineae (CT) and three limb tendons (LT) identified 17,826 differentially expressed genes ($|\log_2 FC| > 2$, FDR < 0.05) (Fig 1A). Periostin (POSTN) ranked among the top ten upregulated genes and exhibited 296-fold higher expression in CT than in LT ($p < 0.01$)(Fig 1B). KEGG and GO enrichment analyses indicated that CT were enriched for biological processes related to positive cell migration, wound response, and differentiation, whereas LT displayed enrichment of apoptotic and negative regulatory pathways (Fig1C-D). Histologically, porcine CT (n = 8) from both healthy and Grade 3 hypertensive pigs maintained comparable cellularity and matrix organization, confirming high intrinsic load tolerance of heart tendons (Fig1E). In the in vitro Cell Strex bioreactor (Fig 2A), porcine CT-derived TSC (n = 6) maintained identical proliferation (Fig 2B), viability (Fig 2C), and migration capacity (Fig 2D) under different uniaxial cyclic loading conditions (0.5Hz for 24 hours, 0%-9% strains). In contrast, porcine IST-derived TSC (n = 6) exposed to 9% tendinopathic cyclic strain at 0.5 Hz for 24 hours showed reduced proliferation ($p < 0.0001$) (Fig 2E), increased apoptosis ($p < 0.0001$) (Fig 2F), and reduced migration ($p < 0.05$) (Fig 2G) compared with the 0% static group. Treatment with recombinant human POSTN (1 µg/mL) under 9% strain significantly improved porcine IST TSC (n=6) proliferation ($p < 0.01$) (Fig 2H), reduced apoptosis ($p < 0.001$) (Fig 2I), and enhanced migration ($p < 0.01$) (Fig 2J) relative to untreated 9% overload groups ($p < 0.001$). In the ex vivo Tendon Strex bioreactor (Fig 3A), porcine IST strips (n = 6) subjected to 9% cyclic strain (0.25 Hz, 8 h/day for 6 days) demonstrated collagen disorganization, heterogeneous fibril diameters, and loss of fibripositors on SEM (Fig 3B) and TEM imaging (Fig 3C). RT-qPCR revealed that porcine IST strips (n = 24) in the 9% tendinopathic group downregulated all four tenogenic markers, Col I a1($p < 0.001$), Tnmd($p < 0.001$), Mkx ($p < 0.01$), and Scx ($p < 0.0001$) significantly compared to 0% static group (Fig 3D). POSTN treatment (1 µg/mL) restored tenogenic gene expression of Col I a1, Scx, and Mkx ($p < 0.05$), while Tnmd showed a non-significant upward trend compared to untreated 9% overloaded porcine IST strips (n = 18) (Fig 3E).

DISCUSSION: Integrated transcriptomic, cellular, and tissue-scale analyses, this study identifies POSTN as a key mediator of tendon resilience. By stabilizing TSC proliferation, limiting apoptosis, and promoting cell migration and tenogenic remodeling under high strain, POSTN bridges cardiac and musculoskeletal tendon biology. Limitations include the use of large-animal tissues rather than human samples and short-term loading models that may not fully recapitulate chronic tendinopathy. The downstream signaling pathways mediating POSTN's protective effects also remain to be elucidated. In conclusion, POSTN enhances tendon resilience by stabilizing cellular viability and promoting regenerative adaptation under overload strain, representing a promising therapeutic target to biologically restore function and prevent degeneration in rotator cuff overuse tendinopathy.

SIGNIFICANCE/CLINICAL RELEVANCE: This work uncovers POSTN as a mechanosensitive molecule that enables tendons to withstand damaging overload—essentially translating the heart tendon's durability to the shoulder tendon. By defining a unifying mechanism of tendon resilience to overuse injuries, it opens a new therapeutic avenue to biologically restore tendon integrity and improve recovery in rotator cuff tendinopathy.

IMAGES AND TABLES:

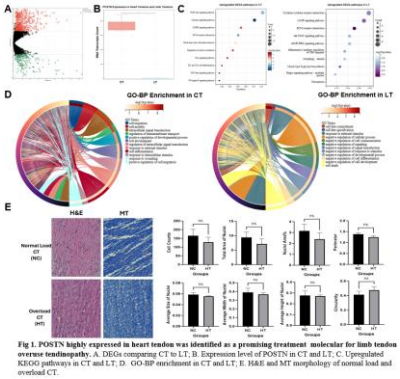

Fig 1. POSTN highly expressed in heart tendon was identified as a promising treatment molecular for limb tendon overuse tendinopathy. A. DEGs comparing CT to LT; B. Expression level of POSTN in CT and LT; C. Upregulated KEGG pathways in CT and LT; D. GO-BP enrichment in CT and LT; E. H&E and MT morphology of normal load and overload CT.

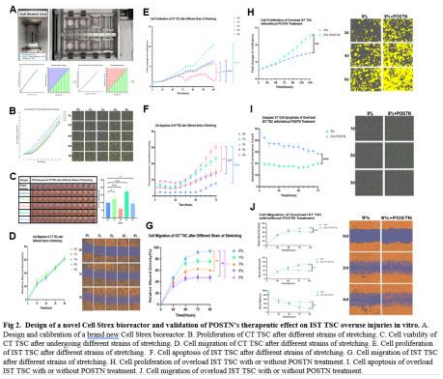

Fig 2. Design of a novel Cell Strex bioreactor and validation of POSTN's therapeutic effect on IST TSC overuse injuries in vitro. A. Design and calibration of a brand new Cell Strex bioreactor. B. Proliferation of CT TSC after different strains of stretching. C. Cell viability of CT TSC after undergoing different strains of stretching. D. Cell migration of CT TSC after different strains of stretching. E. Cell proliferation of IST TSC after different strains of stretching. F. Cell apoptosis of IST TSC after different strains of stretching. G. Cell migration of IST TSC after different strains of stretching. H. Cell proliferation of overload IST TSC with or without POSTN treatment. I. Cell apoptosis of overload IST TSC with or without POSTN treatment. J. Cell migration of overload IST TSC with or without POSTN treatment.

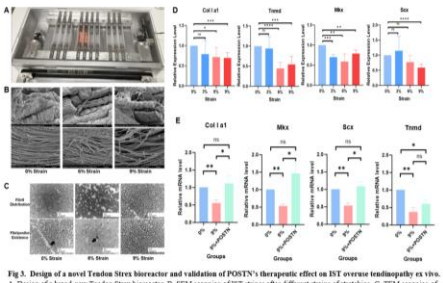

Fig 3. Design of a novel Tendon Strex bioreactor and validation of POSTN's therapeutic effect on IST overuse tendinopathy ex vivo. A. Design of a brand-new Tendon Strex bioreactor. B. SEM scanning of IST stripes after different strains of stretching. C. TEM scanning of IST stripes after different strains of stretching. D. Expression of tenogenic genes in IST stripes after different strains of stretching. E. Expression of tenogenic genes in tendinopathic IST stripes with or without POSTN treatment.