

Tendon-Targeted delivery of drug-loaded nanoparticles to modulate healing

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Introduction: Satisfactory tendon healing following acute injury is marred by a fibrotic response that impairs complete functional recovery. Current approaches in treating tendon injuries involve surgical and physical therapy techniques, however, there is a need for biological augmentation of the healing process to promote regenerative healing¹. Moreover, tendon-specific targeting of systemic pharmacotherapies is limited. Using spatial transcriptomic profiling, we recently identified a spatiomolecular cluster enriched for inflammatory processes that is defined by *Acp5*, the gene that encodes for Tartrate resistant Acid Phosphatase (TRAP)², and demonstrate robust TRAP activity in the healing tendon. As such, we hypothesize that employing a TRAP binding peptide nanoparticle (TBP-NP) delivery system³ will result in high affinity targeting of TRAP⁺ cells in the healing tendon. In addition, we will use this tendon-targeted drug delivery system to blunt S100a4 expression, via delivery of a small molecule transcriptional repressor, Niclosamide. Given the key role for S100a4 in promoting fibrotic tendon healing, we expect that tendon-targeted S100a4 inhibition will promote regenerative tendon healing.

Methods: *PSMA-b-PS NP and TBP synthesis:* Reversible addition-fragmentation chain transfer (RAFT) polymerization was used to synthesize PSMA-b-PS copolymers. A TRAP-binding peptide (TBP) and a scrambled control peptide (SCP) were synthesized and conjugated to PSMA-b-PS copolymers via anhydride ring open chemistry. After conjugation, TBP and SCP functionalized-nanoparticles (TBP-NP, SCP-NP) were loaded with an IR780 dye for tracking in vivo (IVIS), or with Niclosamide for S100a4 knockdown studies. Drug loading efficiency was determined via UV-Vis and HPLC. *Mice:* All studies were approved by the University Committee on Animal Resources. 10 – 12-week-old Female C57Bl/6J mice underwent complete transection and repair of the flexor digitorum longus (FDL) tendon in the hind paw. TBP-NPs, SCP-NPs, and saline were administered retro-orbitally on D7 post injury to assess the uptake and impact of drug-loaded NPs in the healing tendon. Knockdown of S100a4 was determined at D10 post-surgery via qPCR.

Results: *Abundant TRAP⁺ cells are found in the healing tendon during the late inflammatory and early proliferative phases.* No TRAP activity was observed prior to D7 post-repair, however, by D7, several TRAP⁺ cells were observed in the bridging tissue between the tendon stubs, with an increase in TRAP activity at D14 (**Fig1A**). *TBP-NPs effectively target sites of high TRAP activity in vivo.* Although untargeted SCP-NPs demonstrated some accumulation at the repair site, TBP-NPs resulted in significantly higher accumulation (~four-fold vs. SCP-NP, $p < 0.05$), and prolonged retention (14 days), relative to SCP-NPs (**Fig1B-C**). *TBP-NPs are taken up by macrophages.* About 50% of TBP-NPs present in the bridging scar tissue of the tendon were internalized by F4/80+ macrophages after NP administration, identifying them as the primary cells that internalize TBP-NPs. (**Fig1E**). *TBP-NPs loaded with Niclosamide inhibit S100a4 gene expression.* S100a4 is a calcium binding protein which has been implicated in tendon fibrosis⁴. To knockdown S100a4 activity, TBP-NPs were efficiently loaded with a small molecule drug (~80% loading efficiency), Niclosamide (TBP-NP^{Niclosamide}) and delivered to mice 7 days post-op. Significant inhibition of S100a4 gene expression was achieved with TBP-NP^{Niclosamide} systemic delivery 72h after administration (**Fig1F**), establishing an opportunity to modulate tendon healing using this drug delivery system. Ongoing studies are investigating the morphological and biomechanical outcomes of the tendon after treatment with TBP-NP-NEN.

Discussion: Without targeting strategies, small molecules exhibit poor tendon targeting, hence, to enhance drug accumulation at tendon repair sites, a suitable and effective drug delivery system is required. The principal goals of this study were: i) establish that TBP-NPs enhance tendon-targeting of systemic treatment, ii) identify the primary cells that take up TBP-NPs within the tendon, and iii) establish knockdown of S100a4 gene expression during tendon healing using this DDS. While macrophages are primarily associated with phagocytic functions, our co-localization studies of TRAP and TBP-NPs suggest that macrophages exhibit an enhanced affinity for the targeted nanoparticles likely due to TRAP expression, leading to their preferential uptake during the late inflammatory period. S100a4, which is expressed by both macrophages and tenocytes has been implicated in tendon fibrosis. The targeted knockdown of S100a4, facilitated by macrophage targeting, establishes a direct link between cellular manipulation and the subsequent effects on the healing response, which will be determined via functional biomechanical and histological studies. Moreover, the potential to replicate results showing improved tendon healing after 50% knockdown of S100a4 in previously established genetic mouse models⁴ using this translational mechanism is of considerable significance. Collectively, these data demonstrate that TBP-NPs substantially enhance targeting of systemic treatments to the healing tendon and establish that TBP-NPs can be used to deliver a drug to modulate tendon healing due to both high-efficiency targeting and sustained retention of NPs.

Significance: This study establishes a novel means of high-efficiency and specific targeting of the healing tendon via systemic treatment with TRAP-binding-peptide laden nanoparticles. Based on the versatile drug loading chemistry and the formation of uniform spherical NPs with favorable physicochemical properties, this is an innovative platform for small molecule delivery to promote tendon regeneration.

References: ¹Langer R, JP Vacanti. 1993. Science., ²Ackerman et al. Cell Reports. 2023, ³Wang et al. 2017. ACSNano, ⁴Ackerman et al. eLife. 2019.

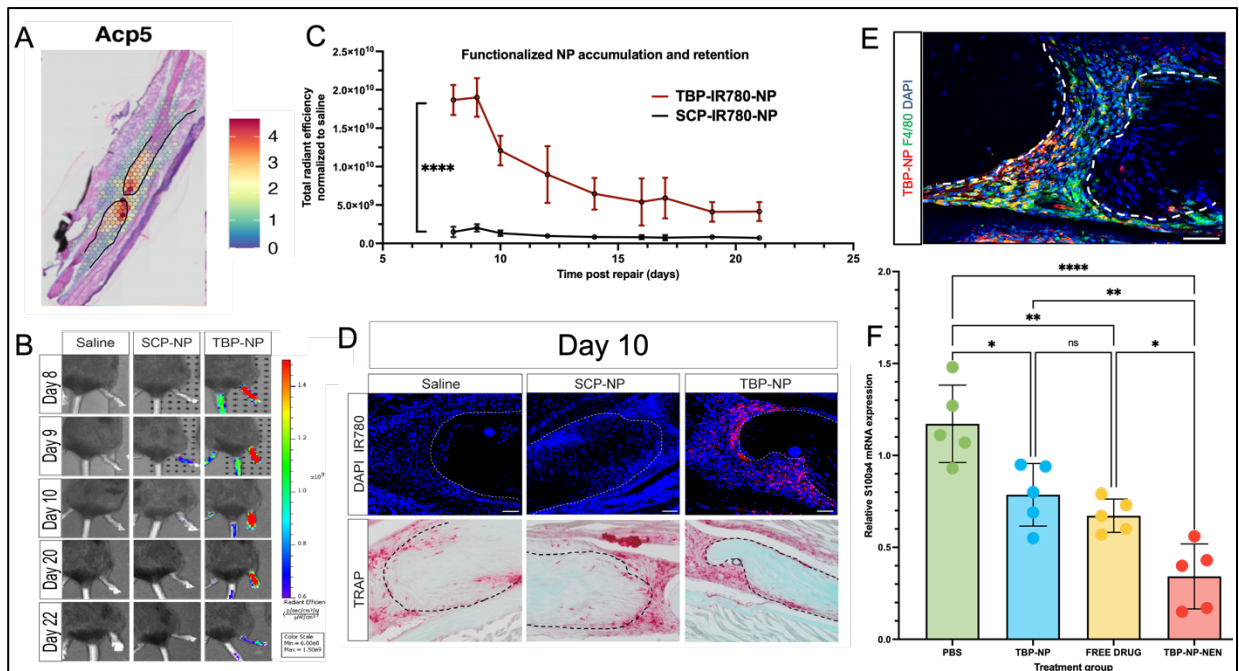


Figure 1. Use of a drug-loaded, peptide-functionalized nanoparticle delivery system to target injured tendon. (A) TRAP staining showing TRAP activity in the tendon. (B) Live animal imaging (IVIS) and (C) quantification showing biodistribution of NPs after D7 treatment. (D) Histological co-localization staining showing preferential targeting of TBP-NPs to TRAP⁺ areas. Scale bar = 200µm. TBP-NPs are internalized by (E) macrophages. Scale bar = 100µm (F) TBP-NP-NEN effectively inhibits S100a4 gene expression in the tendon. N = 5, mean +/- SD. $P < 0.05$.