Development of a Minimally Invasive Drug Delivery Platform for the Effective Treatment of Tendon Injuries

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INTRODUCTION: Tendon injury is a common and devastating orthopaedic condition. Many injured patients sustain long-term pain and physical disability due to excessive inflammation and unsatisfactory tendon self-repair. Several growth factors and stem cell-derived agents have shown great potential to improve tendon healing by limiting inflammation and stimulating endogenous tendon stem/progenitor cell proliferation and tenogenic differentiation.[1-3] However, the translational application of these agents has been hindered due to the challenges of drug delivery. Tendon healing is a lengthy and complicated process requiring sustained and stage-specific treatments. Single-dose administration during surgical repair can't fulfill the purpose. The tendon is a dense fibrous tissue underneath the skin. The efficiency of topical administration is limited by the abilities of individual drugs to penetrate and permeate through the skin, tendon sheath and fibers. Extracellular vesicles (EVs) are a versatile natural drug carrier capable of encapsulating diverse therapeutic agents including small molecule compounds, miRNAs, mRNAs, and proteins. Moreover, EVs from adipose-derived stem cells (ASCs) were recently found to be taken by many tendon cells after intradermal injection to the nerve-free upper dermis. Because the minimally invasive intradermal route bypasses the skin barrier, and ASC EVs can efficiently penetrate through the tendon sheath and fibers and target tendon cells, this study developed and characterized a new delivery platform consisting of a dissolvable microneedle (DMN) patch for minimally invasive drug administration and ASC EVs as a tendon-targeting drug carrier. The new platform is expected to provide sustainable and stage-specific treatments to injured tendons by several painless administrations.

METHODS: This study was approved by IACUC. Tendon cells were isolated from canine extrasynovial flexor tendons. EVs were prepared from an EV-free conditional medium of ASC culture via differential centrifugation. The connective tissue growth factor (CTGF) mimics 4-PPBP maleate (4-PPBP) and Oxotremorine M (Oxo-M) or fluorescent model drug propidium iodide (PI) were loaded into ASC EVs via sonication. The resulting EVs were characterized via dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). EV entrapment efficiency (EE) was determined by HPLC/UV analysis. The functionality of encapsulated Oxo-M and 4-PPBP was determined in isolated tendon cells by a MTT assay for cell proliferation and quantitative PCR for tenogenic gene expression. DMN patches, consisting of an array of cone-shaped dissolvable polyvinylpyrrolidone microneedles (600 μm long) with or without ASC EVs and an insoluble polystyrene back layer, were fabricated with a silicone mold. Microneedle dissolution and EV release were assessed in PBS in vitro. The drug delivery properties of the DMN patch were evaluated in adult *ScxGFP* mice of both sexes. DMN patches containing PI-laden EVs (PI-EVs) were pushed into the skin covering the Achilles tendon and then removed after 30 minutes. The biodistribution of PI was monitored via live fluorescence imaging at varied time points after patch removal and postmortem histological analysis. Changes in DMN geometry and fluorescence were evaluated via scanning electron microscopy (SEM), μCT, and epifluorescence microscopy. Paired student's t-test and one-way ANOVA followed by Tukey's tests were used for two-group and multigroup comparisons (n=3-6/group).

RESULTS: Conditions for drug encapsulation in EVs were optimized to achieve an encapsulation efficiency of 13.0% and 45.3% for Oxo-M and 4-PPBP and a particle recovery rate of 74.9% and 42.4% for EVs loaded with Oxo-M and 4-PPBP, respectively. The bioactivity of encapsulated drugs in tendon cells were assessed in vitro. Results showed that encapsulated Oxo-M induced 1.4 times greater proliferation than naive EVs after 48-hour treatment ($p<0.05$). Combined application of encapsulated Oxo-M and 4-PPBP increased the expression level of *Scx* in tendon cells by over 7-fold ($p<0.05$ vs untreated cells). Drug-laden ASC EVs were incorporated into the needle portion of DMN patches. The dissolution of EV-containing microneedles in PBS was confirmed by micro-CT and SEM (Fig. 1A), showing a ~31.6% reduction in needle height within 24 hours. As a result, nearly all incorporated EVs were released from the DMN patch within three hours (Fig. 1B). PI was used as a fluorescent model drug to track drug distribution following DMN patch administration (Fig. 2A). Live fluorescence imaging of treated mice detected PI signals (red) at the Achilles tendon region of the limb treated with an EV-PI containing DMN patch but not an EV-PI-free patch 5 hours after administration (Fig. 2B, circled area). Post-mortem imaging of tendon sections 4 days after PI administration detected extensive PI signals preferentially accumulated at GFP-expressing tendon cells, thus supporting the use of DMN patch and ASC EVs for minimally invasive delivery of tendon drugs.

DISCUSSION: This study developed an intradermal drug delivery platform consisting of an intradermal DMN patch and tendon-targeting ASC EVs. Our results demonstrated that both hydrophilic Oxo-M and hydrophobic 4-PPBP can be successfully loaded into ASC EVs and promote tendon cell proliferation and tenogenic gene expression. Results from our live imaging and histological analysis further proved the use of the intradermal DMN patch to deliver tendon drugs. It remains to be determined whether drugs delivered via the new platform can achieve an effective dose within desired treatment window for enhanced tendon healing.

SIGNIFICANCE: Successful development of the minimally invasive and self-administrable drug delivery system will enable sustained and stage-specific tendon drug delivery for enhanced tendon healing, improve patient compliance, and benefit many injured patients.

REFERENCES: [1] Shen H, et al. J Orthop Res. 2020;38:117-127. [2] Shen H and Lane R. Stem Cells. 2023;41:617-627. [3] Shen H, et al. Sci Rep. 2018;8(1):11078.

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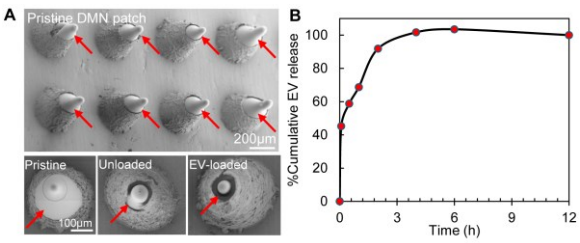

Figure 1. (A) Representative SEM images of DMNs before (pristine) and after immersed in PBS for 24 hours. Red arrows point to the PVP microneedles. (B) Cumulative release of ASC EVs from a DMN patch.

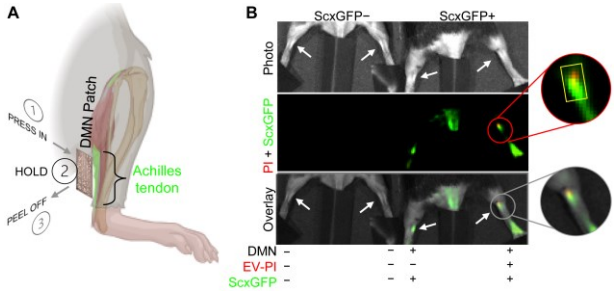

Figure 2. (A) Intradermal application of a DMN patch over a mouse Achilles tendon. (B) Representative live fluorescence images of mouse hindlimbs 5 hours after indicated treatments. White arrows point to the Achilles tendon. EV-PI, EV-encapsulated PI.