Losartan-Loaded Nanofibers Attenuate Postoperative Adhesion and Enhance Tendon Healing After Tendon Repair

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Background: Tendon injuries caused by trauma, overuse, or degeneration, frequently lead to adhesions and re-ruptures despite advances in surgical repair technique and rehabilitation. These complications primarily result from fibrotic scar tissue formation, which has inferior mechanical properties compared to native tendon. Transforming growth factor-beta 1 (TGF-β1) plays a key role in tendon healing but its overexpression drives fibrosis and adhesion through promoting excessive growth of myofibroblasts. We developed peptide amphiphile (PA) nanofibers for sustained local delivery of losartan, previously demonstrating their effectiveness in inhibiting fibrocartilage regeneration and promoting hyaline-like cartilage regeneration[1,2].
In vitro experiments have since shown that these losartan-encapsulated PA nanofibers effectively suppress renin-angiotensin-aldosterone system (RAAS) and TGF-β/Smad signaling in tenocytes[3]. By blocking angiotensin II type 1 receptor, losartan reduces TGF-β1 expression and downstream Smad2/3 phosphorylation, decreasing extracellular matrix synthesis—including type I (COL1) and type III collagen (COL3) and fibronectin—and alpha-smooth muscle actin (α-SMA), a marker of myofibroblast and fibrosis. This leads to inhibition of fibrotic gene expression and excessive matrix deposition, preventing scar formation. Building on these *in vitro* findings, the present *in vivo* study evaluates the ability of losartan nanofibers to reduce adhesion and fibrosis after tendon repair, aiming to improve functional healing outcomes.
Methods: Losartan-encapsulated PA nanofibers combined with hyaluronic acid (HA) were prepared to form a gel and enable sustained local drug delivery. Losartan release kinetics from the nanofibers, both alone and combined with HA, were evaluated *in vitro* to characterize sustained drug release. For biodistribution, HA-nanofibers labeled with cyanine 7 dye were implanted around the Achilles tendon repair site in four rats, and fluorescence imaging was performed at multiple time points up to 21 days using IVIS Spectrum.
Thirty rats underwent Achilles tendon transection and repair using a modified Kessler suture technique and were randomly divided into five groups (6 rats per group): i) control (PBS 0.5ml), ii) losartan solution group (1mg/ml losartan solution 0.5ml), iii) nanofiber (without losartan, 0.5ml), iv) early losartan nanofiber group (2 mg/ml losartan nanofiber, 0.5ml applied at surgery), and vi) late losartan nanofiber group (2 mg/ml losartan nanofiber, 0.5ml applied 2 weeks postoperatively via mini-open incision). The control and losartan solution groups were administered at 0, 2, 4 weeks postoperatively. Rats were sacrificed 6 weeks post-surgery. Adhesion was macroscopically assessed following Tang et al.'s criteria; samples were harvested for histological evaluation. Harvested tendons were stained with H&E, Masson's Trichrome, and Alcian blue. Histological evaluation used Movin and Bonar scoring systems by two blinded observers. Immunohistochemistry for COL1 and COL3 and αSMA was performed with specific antibodies. Positive staining areas were quantified using ImageJ from multiple random fields around the repair site.
Statistical analysis: All data were analyzed using GraphPad Prism version 9.4.0. Results are presented as mean ± standard deviation, with individual replicates shown as dots. Groups were compared using one-way or two-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was set at $p < 0.05$.
Results: *In vitro* evaluation of losartan release kinetics from the HA-nanofiber demonstrated sustained delivery, with 50–60% of the drug released over three weeks. Cyanine 7-labeled HA nanofibers implanted around the Achilles tendon repair site remained detectable for up to 14 days post-surgery, with fluorescence gradually declining by day 21, indicating progressive nanofiber degradation and clearance (**Fig.1**). Losartan-encapsulated nanofibers significantly reduced macroscopic tendon adhesions compared to controls, with both early and late application groups showing milder adhesion severity. Histological analyses using Movin and Bonar scores revealed improved tendon tissue organization and cellular morphology in all losartan-treated groups versus controls (**Fig.2**). Immunohistochemistry showed increased COL1 expression and decreased COL3, especially with early losartan nanofiber application, resulting in a higher COL1/COL3 ratio indicative of enhanced matrix remodeling (**Fig.3**). Assessment of fibrosis via αSMA staining demonstrated significant suppression of myofibroblast activity in both tendon core and surface scar tissue in all treatment groups. Notably, the late losartan nanofiber group exhibited lower αSMA levels than unloaded nanofibers, highlighting the benefits of sustained drug release (**Fig.3**).
Discussion: This study demonstrated that losartan-encapsulated PA nanofibers effectively reduced fibrotic adhesion and scar formation following Achilles tendon repair in a rat model. Our findings indicate that the local delivery of losartan via a PA-based nanofiber scaffold provides sustained inhibition of TGF-β signaling, leading to improved collagen remodeling, decreased α-SMA expression, and a favorable COL1/COL3 ratio – collectively suggesting both reduced fibrosis and enhanced matrix organization.
Significance: Sustained local delivery of losartan via nanofibers shows promise as a therapeutic strategy to prevent adhesion and improve tendon healing quality.
Reference: 1. Yamaura, K., et al. Front Bioeng Biotechnol. 2023. 2. Yamura K., et al. ORS 2024. 3. Mukohara S., et al. ORS 2025.
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Figure 1

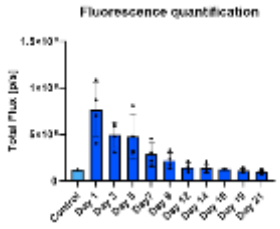

Figure 2

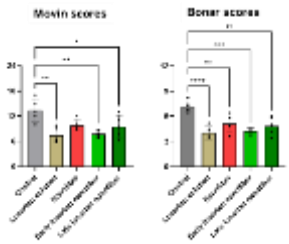

Figure 3