Liquid Poly-N-Acetyl Glucosamine (sNAG) Improves Achilles Tendon Healing in a Rat Model

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Introduction

- The Achilles tendon, the strongest and largest tendon in the body, is frequently injured.
- Even after surgical repair, patients can have long-term deficits in function [1].
- Various forms of biological augmentation have been utilized to improve tendon repair [2].
- Poly-N-acetyl glucosamine (sNAG) polymer has been shown to increase the rate of healing of venous leg ulcers [3].
- Use of this material also improved tendon-to-bone healing in a rat model of rotator cuff injury and repair [4].
- Whether this nanofiber material, in a minimally invasive injectable formulation, could improve soft tissue tendon healing after Achilles injury is unknown.
- **Objective**: To investigate the healing properties of an injectable sNAG liquid in a rat partial Achilles tear model.
- **Hypothesis**: sNAG would improve tendon healing as measured by improved mechanical properties and cellular morphology.

Materials and Methods

Experimental Design

- 32 adult male Sprague-Dawley rats; IACUC-approved
- Animals underwent a partial-width, full thickness injury (1.5 mm biopsy punch) through the right Achilles tendon [5] .
- After injury, animals received either:
  - 10 µl of 0.9% saline (control group) (n=16)
  - 10 µl of 20 mg/ml sNAG polymer liquid (sNAG group) (n=16)
- All animals:
  - were allowed normal cage activity after surgery, without immobilization
  - received repeat saline or sNAG injections at the injury site percutaneously at one and two weeks post-injury
  - were sacrificed three weeks post-injury

Tendon Mechanical Testing

- Animals (n=10/group) were frozen at -20°C and later thawed for dissection and mechanical testing.
- The Achilles tendon and foot complex was dissected and the calcaneus potted in poly(methyl methacrylate) (Fig. 1).
- In a 37°C PBS bath and in a physiologic orientation, Achilles tendons were subjected to preloading, stress relaxation at 6% strain, dynamic frequency sweeps, and fatigue cycling under load control until failure.

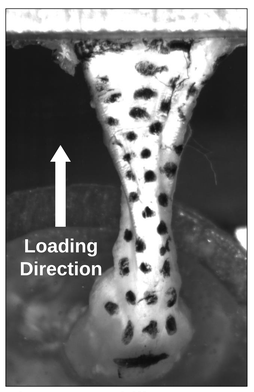

Figure 1. Achilles mechanical testing. Calcaneus-foot complex in PMMA with stain dots for optical strain tracking.

Histology

- At time of sacrifice, the Achilles-calcaneus complex was harvested and paraffin processed (n=6/group).
- Histological analysis included semi-quantitative morphological assessments and quantitative collagen organization analysis.

Statistics

- Mechanical testing and collagen fiber organization data were evaluated using two-tailed t-tests after confirming data normality.
- Semi-quantitative histological comparisons were made using Mann-Whitney U tests.
- Significance was set at $p<0.05$ and trends at $p<0.1$.

Results

Mechanical Properties

- At three weeks post injury, there was no difference in tendon cross-sectional area (not shown).
- Tendon stiffness was improved with sNAG treatment (Fig 2A), but modulus was not different between groups (Fig 2B).
- Two-way ANOVA demonstrated an increase in dynamic modulus with sNAG treatment across frequencies (Fig 2C, $p=0.004$ for treatment; no pair-wise differences) and tanδ, a measure of force dissipation, was not different (not shown).
- Fatigue testing demonstrated increases in tendon secant stiffness (Fig. 2D) and tangent stiffness (Fig. 2E) throughout fatigue life for sNAG-treated tendons compared to controls.
- There was no difference in cycles to failure (Fig. 2F), or other properties measured (not shown).

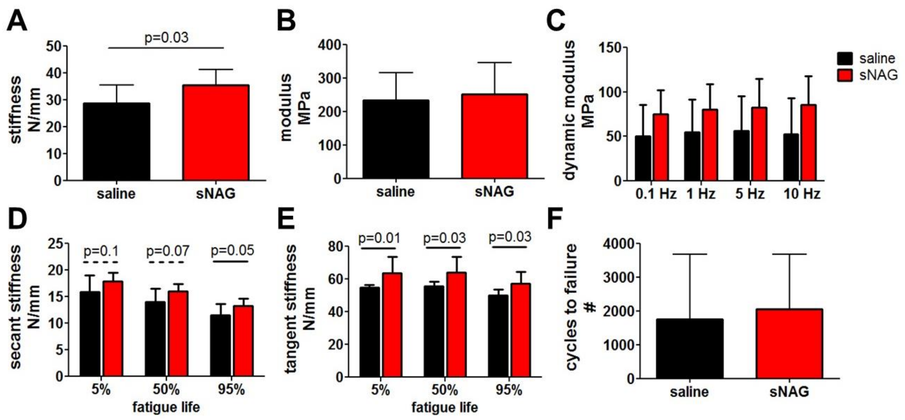

Figure 2. Mechanical Properties. Three weeks after injury, sNAG treated tendons had (A) increased stiffness, (B) no change in modulus, (C) increased dynamic modulus across testing frequencies, (D) increased secant stiffness, and (E) increased tangent stiffness. There were no changes in (F) cycles to failure between groups. Data shown as mean+SD.

Histologic Observations

- Semi-quantitative grading did not demonstrate differences in cell density (Fig. 3A) or cell shape (Fig. 3B) at the injury region.
- Collagen alignment in this region was also not different between groups (Fig. 3C).
- Representative images of the injury region for both groups are shown in Figure 3D.

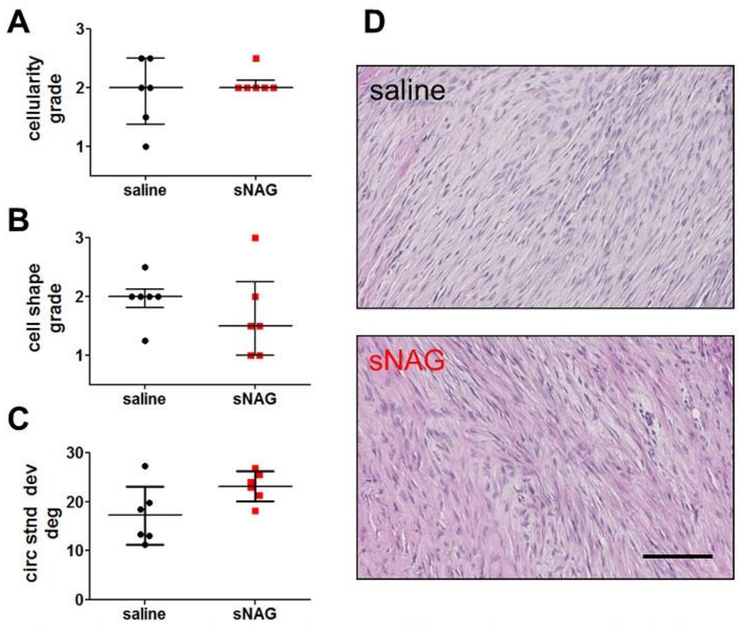

Figure 3. Histological Properties. There were no differences between groups for (A) cellularity, (B) cell shape, or (C) collagen alignment. Representative images of the injury region are shown in (D). Data represented as median±IQR in A and B, and as mean±SD in C. Scale bar in D: 100 µm.

Conclusions

- This study investigated the effects of repeated sNAG polymer application on tendon healing after a full thickness, partial width Achilles injury.
- Although several parameters did not exhibit differences between treatment groups, other results demonstrate that **sNAG has a positive effect on rat Achilles tendon healing** at three weeks .
- Quasistatic testing demonstrated **increased tendon stiffness** with sNAG treatment, which continued during fatigue cycling, as shown in increased tangent and secant stiffness across fatigue life.
- Increased dynamic modulus also suggests **improved viscoelastic properties** with sNAG treatment.
- Importantly, use of this material did not have any negative effects on any measured parameter.
- Previous studies suggest that this material may mitigate pain after rotator cuff injury [4]. Functional testing such as gait assessment could potentially evaluate the use of this material for painful Achilles tendonitis [6].
- These results support further study of this material as a **minimally invasive treatment modality** for tendon healing.

References & Acknowledgments

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Funding was provided by Marine Polymer Technologies and the Penn Center for Musculoskeletal Disorders (P30 AR069619). We thank Stephanie Weiss and Peter Chan or their assistance.