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

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Introduction

The Achilles tendon, while the strongest and largest tendon in the body, is frequently injured. Even after surgical repair, patients risk re-rupture and typically have long-term deficits in function, with a low rate of return to pre-injury levels of activity.[1] Various forms of biological augmentation have been utilized in an attempt to improve tendon repair.[2] Poly-N-acetyl glucosamine (sNAG) polymer has been shown to increase the rate of healing of venous leg ulcers, with an 86% success rate clinically.[3] Additionally, use of this material improved tendon-to-bone healing in a rat model of rotator cuff injury and repair.[4] However, whether this nanofiber material, in an injectable liquid formulation, could improve soft tissue tendon healing after Achilles injury is unknown. Therefore, the purpose of this study was to investigate the healing properties of sNAG containing membranes in a rat partial Achilles tear model. We hypothesized that sNAG would improve tendon healing as measured by improved mechanical properties and cellular morphology.

Methods

Study Design

32 adult male Sprague-Dawley rats (400-450g) were used in this IACUC-approved study. All animals underwent a partial-width, full thickness injury using a 1.5 mm biopsy punch through the right Achilles tendon as described.[5] After injury, animals were randomized into two groups, receiving either 10 µl of 0.9% saline (control group) or 10 µl of 20 mg/ml sNAG polymer gel (sNAG group). Animals were allowed normal cage activity after surgery, without immobilization. Animals received repeat saline or sNAG injections at the site of the injury through the skin at one and two weeks post-surgery. All animals were sacrificed three weeks after injury.

Ex Vivo Assessments

The Achilles-calcaneus complex was immediately harvested and processed for histological analysis including quantitative collagen fiber organization analysis (n = 6/group). All other animals (n = 10 per group per time point) were frozen at −20°C and later thawed for dissection and mechanical testing. For testing, the Achilles tendon and foot complex were dissected and the calcaneus was potted in poly(methyl methacrylate). While immersed in 37°C phosphate-buffered saline and in a physiologic orientation, the Achilles tendons were gripped and subjected to a mechanical loading protocol consisting of: preloading, stress relaxation at 6% strain, dynamic frequency sweeps, and fatigue cycling under load control until specimen failure.

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 $p < 0.05$ for all comparisons.

Results

Mechanical properties

At three weeks after injury, there was no difference in tendon cross-sectional area (not shown). Tendon stiffness was improved with sNAG treatment (Fig 1A), but modulus was not different between groups (Fig 1B). Frequency sweeps demonstrated an increase in dynamic modulus across tested frequencies (Fig 1C), but tanδ, a measure of force dissipation, was not different (not shown). Fatigue testing demonstrated increases in tendon secant stiffness (Fig. 1D) and tangent stiffness (Fig. 1E) throughout fatigue life for sNAG-treated tendons compared to controls. There was no difference in cycles to failure (Fig. 1F), or other properties measured (not shown).

Histologic observations

Semi-quantitative grading did not demonstrate differences in cell density (Fig. 2A) or cell shape (Fig. 2B) at the injury region. Collagen alignment in this region was also not different between groups (Fig. 2C). Representative images of the injury region for both groups are shown in Figure 2D.

Discussion

This study investigated the effects of repeated sNAG polymer application on tendon healing after partial Achilles injury. Although several parameters did not exhibit differences between

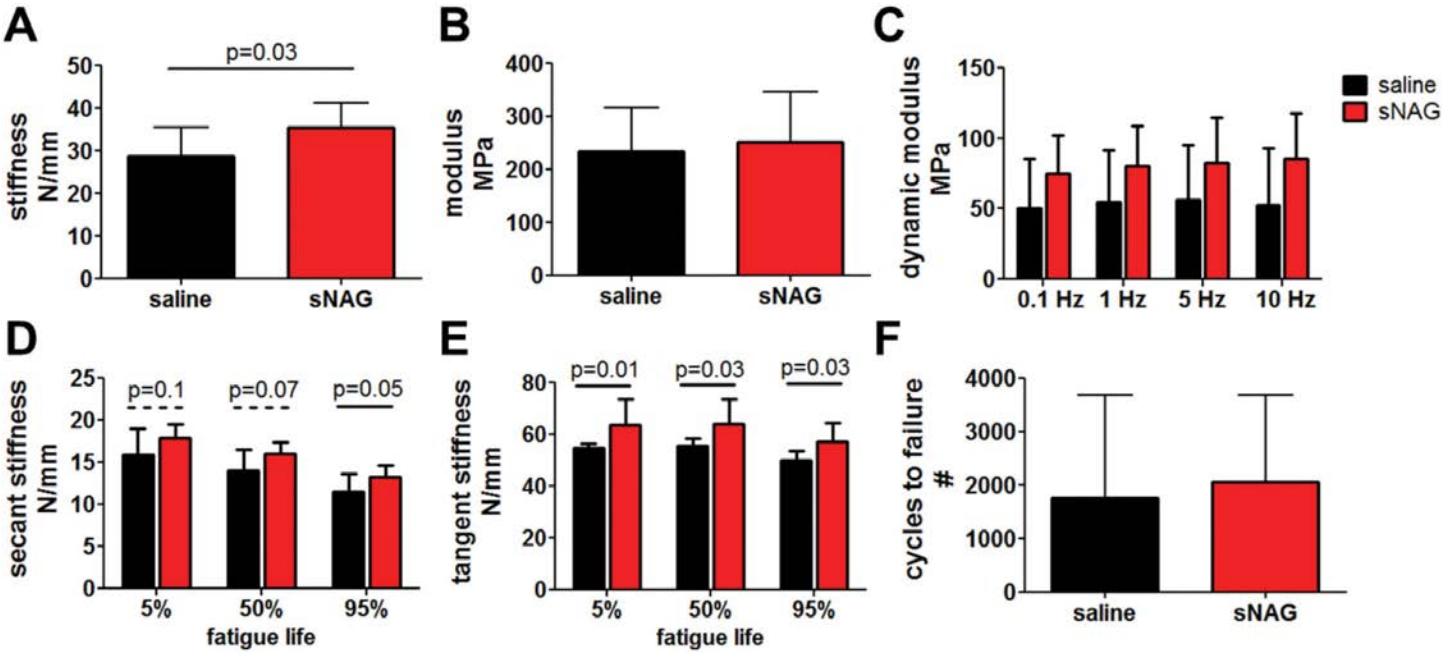

Figure 1. Mechanical Properties. Three weeks after injury, sNAG treated tendons had **(A)** increased stiffness; **(B)** no change in modulus; **(C)** increased dynamic modulus across testing frequencies; **(D)** increased secant stiffness and; **(E)** increased tangent stiffness. There were no changes in **(F)** cycles to failure between groups. Data shown as mean+SD.

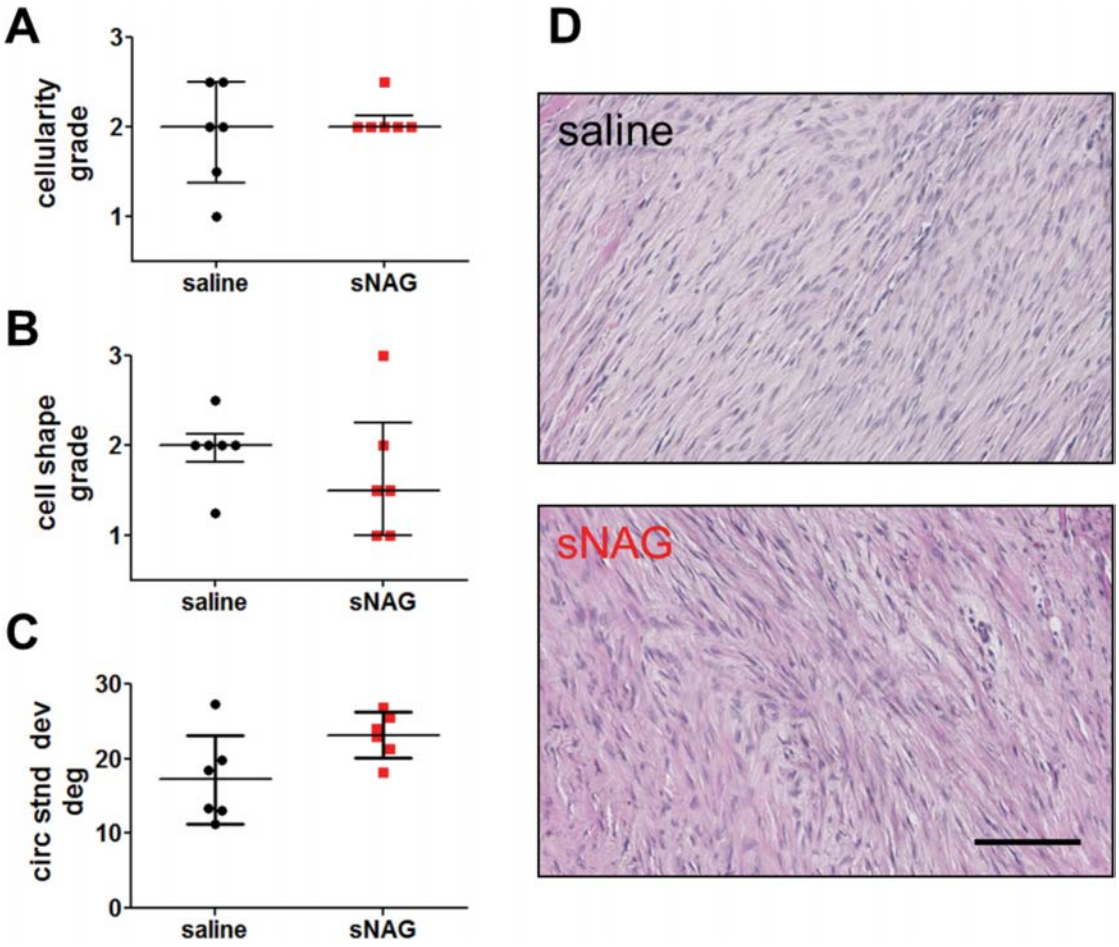

Figure 2. Histological Properties. There were no differences between groups for **(A)** cellularity; **(B)** cell shape; or **(C)** collagen alignment. Representative images of the injury region are shown in **(D)**. Data represented as median±IQR in A and B, and as mean±SD in C. Scale bar in D: 100 μm.

treatment groups, other results demonstrate that sNAG has a positive effect on rat Achilles tendon healing at three weeks after a full thickness, partial width injury. 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 might be valuable, potentially expanding the use of this material as a less invasive treatment for painful Achilles tendonitis.[6] Additionally, dosage studies and number of repeated sNAG injections may optimize the use of sNAG for soft tissue tendon healing. Finally, studies to elucidate the mechanism of action for the changes identified are important.

Significance

Repeated injections of sNAG polymer improve Achilles tendon properties after partial tear. These results support further study of this material as a minimally invasive treatment modality for tendon healing.

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