

Enhancement of Acute Tendon Repair Using Chitosan Matrix

Eitan Melamed, MD, Bryan G. Beutel, MD, and Dror Robinson, MD, PhD

Abstract

Structural failure of rotator cuff repairs has been attributed to multiple factors, including poor repair tissue quality and poor tendon–bone integration. Chitosan gel has been shown to facilitate scarless healing of soft tissues.

In the study reported here, we hypothesized that use of a chitosan gel would improve the morphologic appearance of acute rotator cuff repair in a rat model after 12 weeks. Forty Wistar rats were used. In each case, bilateral tenotomy of the supraspinatus tendon was performed, followed by acute repair with sutures. The left shoulder served as a suture-only control, and the right shoulder was augmented with a chitosan gel applied between the ends of the tendon. Histologic analyses were performed to determine the functional and anatomical characteristics of the repair immediately after the operation and 3 days and 1, 2, 4, 6, 8, and 12 weeks after surgery.

In the gel-augmented specimens, number of fibroblasts and amount of repair tissue were increased. Compared with the controls, these specimens showed minimal evidence of monocytic infiltration or inflammatory response around the matrix. Structural properties of the augmented shoulder, including pennation angles and fatty atrophy, were significantly improved.

These study results showed that use of a chitosan matrix can enhance biological repair of rotator cuff tendons in a rat model.

that changes in muscle fiber orientation (the pennation angle) can cause mesenchymal stem cells to migrate to the interface between muscle fibers and the region of fatty infiltration of the muscle.² Understanding the factors involved in muscle degeneration and atrophy, and in fatty infiltration, may lead to treatments that improve outcomes for patients with massive RCTs. One proposed treatment involves placing continuous mechanical traction on the ends of the torn tendon.² Findings from this research have indicated that acute tears that become chronic tears are typified by inelasticity and poor function of the muscle–tendon unit. It is therefore important to develop a method that speeds tendon healing without causing the muscle fiber atrophy and pennation angle changes that lead to fatty atrophy, which appears to be an irreversible structural change.

On the basis of the theory that adding mesenchymal cells may improve tendon healing, investigators have studied use of transcription factors (eg, scleraxis) specific to tendogenesis in the embryonal stage.^{3,4} Nevertheless, certain transcription factors are associated with formation of fibrocartilage in higher concentrations.⁴ Moreover, decalcified bone matrix increases cartilage formation when added to the tendon repair site.⁵ Cartilage formation, however, is associated with poorer functional results.⁶ Thus, there is a need for a method that facilitates faster tendon healing with higher quality tissue formation and less muscle atrophy.

Chitosan, a linear polysaccharide, is associated with scarless healing of soft tissues and prevention of adhesion formation both intraperitoneally and during tendon healing after surgery.^{7,8} Chitosan tends to precipitate in physiologic pH, thereby mitigating its potency. Fortunately, a chitosan solution that does not precipitate in physiologic conditions was recently developed.⁹ The solution's lack of precipitation, coupled with its in situ gelling, allows it to adhere to the repair site long enough to take effect. These characteristics could allow for intimate contact between gel and tendon, facilitating guided-tissue regeneration and preventing adhesion of the rotator cuff to surrounding tissue. By contrast, other biological agents (eg, platelet-rich plasma) are administered as fluid rather than gel and are therefore more susceptible to diffusing from the repair site, mitigating their effects. Thus, chitosan gel is fairly unique among agents.

Rotator cuff tears (RCTs) are common tendon injuries that can cause chronic pain and severe functional disability. Massive RCTs do not heal spontaneously and, in many cases, result in poor clinical outcomes. Specifically, muscle atrophy and fatty infiltration correlate with poor outcomes after surgical repair.¹ Fatty infiltration of the rotator cuff is a common phenomenon that can lead to permanent structural alterations within the tendon. It has been suggested

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In the study reported here, we histologically investigated whether a chitosan gel would help improve healing of rotator cuff tendon (acute supraspinatus) tears in a rat model.

Materials and Methods

Supraspinatus Surgical Model

Forty Wistar rats, each weighing between 300 and 400 g, were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee at Rabin Medical Center in Petah Tikva, Israel. The rats were anesthetized with ketamine 90 mg/kg and xylazine 10 mg/kg, both administered intramuscularly, and anesthesia was prolonged as needed with 2% isoflurane, administered by nose cone. The skin was incised 5 cm along the upper back following the midline of the spine. The resulting skin flaps were retracted and the scapula exposed. Careful blunt dissection allowed visualization of the rotator cuff and the trans-scapular arch. A full-thickness incision of the supraspinatus tendon was then made 2 mm distal to the arch. This procedure was performed on both shoulders. For the right supraspinatus tendon, a bioabsorbable chitosan-hydrochloric acid solution (>70% de-acetylated chitosan, molecular weight of 600 kDa; Hepepe Medical Chitosan GmbH, Halle, Germany) was sterilely applied to the ends of the tendon (total volume, 0.5 mL) and automatically gelled in situ by heating to about 37°C (rat's internal body temperature). The tendon ends were subsequently approximated with a single 4-0 Prolene suture (Ethicon, Somerville, New Jersey). The left shoulder (tendon repaired with suture only) served as a control.

The rats were housed for a maximum of 12 weeks after surgery. They were sacrificed (in groups of 5 each) 2 hours, 3 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, and 12 weeks after surgery. After each rat was sacrificed, both shoulder girdles were harvested, and the sutures were removed from the supraspinatus tendons.

Histologic Analysis

After routine fixation with 4% formalin for 48 hours and decalcification with 10% ethylenediaminetetraacetic acid (EDTA) for 3 weeks, the specimens were sectioned with a microtome blade. Care was taken to ensure the plane of the microtome blade was parallel with the longitudinal plane of the supraspinatus muscle and tendon to allow for evaluation of pennation angle. Hematoxylin-eosin staining and Masson trichrome staining were subsequently performed.

A variety of histologic measurements were obtained with use of ImageJ software (US National Institutes of Health). Percentage of fibrous tissue was determined by examining the slides at low magnification fields (×25) at the tendon healing site. Three such fields were evaluated per specimen. The fibrous tissue was circled manually, and percentage of tissue area was assessed and compared with total region of interest. Cellularity was carefully outlined and measured as percentage of total tendon area occupied by cells. Fatty atrophy was defined as either present or absent. Muscle fiber diameter was defined as average diameter of 10 muscle fibers measured within 2 mm

of the tendon laceration site. Inflammatory cell collections were defined as either large (>100 μm in diameter) or small (<100 μm in diameter) and were dichotomized to either present or absent. Pennation angle was defined as average angle between muscle fibers and longitudinal axis of supraspinatus muscle and tendon unit. Ten fibers proximal to and within 2 mm of the laceration site were randomly selected, measured, and averaged.

Statistical Analysis

Statistical analysis was performed with Analyse-it 2.20 for Microsoft Excel 2010 (Analyse-it Software, Leeds, United Kingdom). Data were initially analyzed with the Kolmogorov-Smirnov test to assess for normality of distribution. The t test was used to compare continuous variables when the data were normally distributed and the Mann-Whitney test when the data were not normally distributed.

Results

All tendons (both groups) healed within 12 weeks. Generally, the tissue formed at the repair site exhibited a mixture

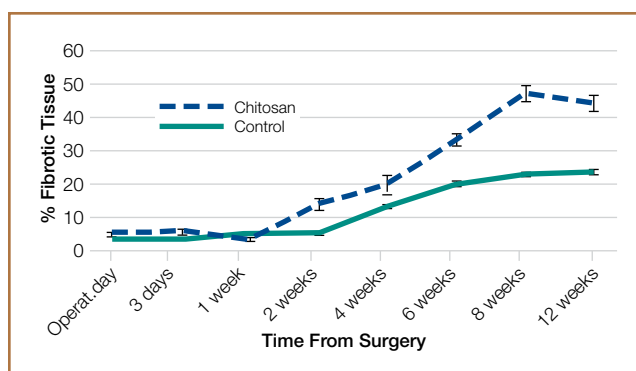


Figure 1. Fibrous tissue formation over time: means and standard deviations at each time point. “Operat. day” refers to sacrifice 2 hours after surgery.

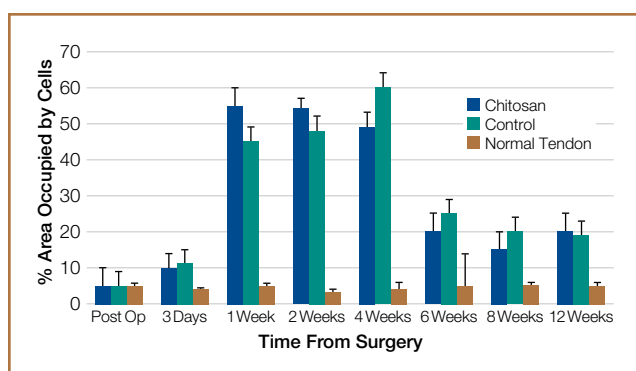


Figure 2. Cellularity at tendon repair sites over time: means and standard deviations at each time point. “Post op” refers to sacrifice 2 hours after surgery.

of tenocyte-like cells (fibrotic tissue) and granulation tissue without clear orientation. As noted in **Figure 1**, the tendons treated with chitosan had more fibrotic tissue (overall mean, 21.5%) relative to the control group (mean, 12.3%), and the difference was significant ($P = .003$). The most notable differences were found at time points later than 1 week after surgery. In addition, amount of cellularity (**Figure 2**) was higher in chitosan-treated tendon and control tendon than in the normal, uninjured adjacent tendon at all time points ($P < .001$). Chitosan-treated tendons had significantly higher cellularity than untreated control tendons from 1 to 2 weeks ($P < .001$), and control tendons were significantly hypercellular compared with chitosan-treated tendons from 4 to 8 weeks ($P < .001$), but both groups exhibited similar cellularity by 12 weeks ($P > .05$). Fatty atrophy was found at significantly higher rates in control rats than in chitosan-treated rats ($P = .001$; **Table**). Furthermore, as noted in **Figure 3**, muscle fiber diameter decreased in both groups after injury ($P < .001$).

Figure 4 shows that the amount of inflammatory collections was significantly smaller in the chitosan-treated group than in the control group over the course of the study ($P = .01$). In addition, pennation angle steadily decreased in the control group throughout the study period, whereas it transiently decreased in the chitosan-treated group (until 2 weeks) before returning to its immediate postoperative level by 12 weeks (**Figure 5**). Overall, the chitosan-treated group maintained a higher pennation angle than the control group did ($P < .001$).

Discussion

RCTs affect more than 40% of patients over age 60 years and are a common cause of debilitating pain, reduced shoulder function, and weakness.¹⁰ Thirty thousand to 75,000 rotator cuff repairs are performed annually in the United States.¹¹ Although the best treatment for this disorder remains a topic of debate, arthroscopic and (when necessary) open surgical repair is the accepted gold standard for the treatment of tears that do not improve with conservative management. Despite advances in

the surgical treatment of these tears, the surgical failure rates are high (range, 20%-90%), with failures attributed to factors beyond patient age, tear size and chronicity, muscle atrophy and degeneration, tendon quality, repair technique, and post-

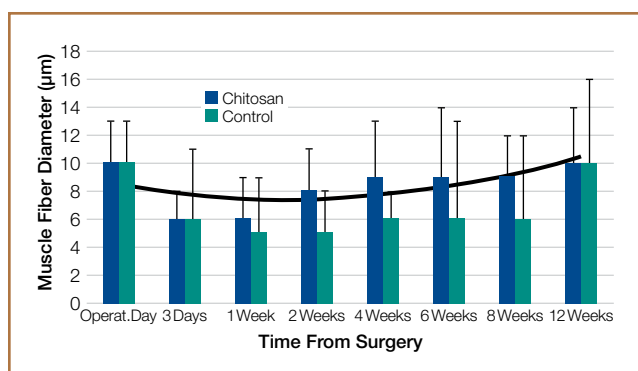


Figure 3. Muscle fiber diameter over time: means and standard deviations at each time point, with gross trend line. “Operat. day” refers to sacrifice 2 hours after surgery.

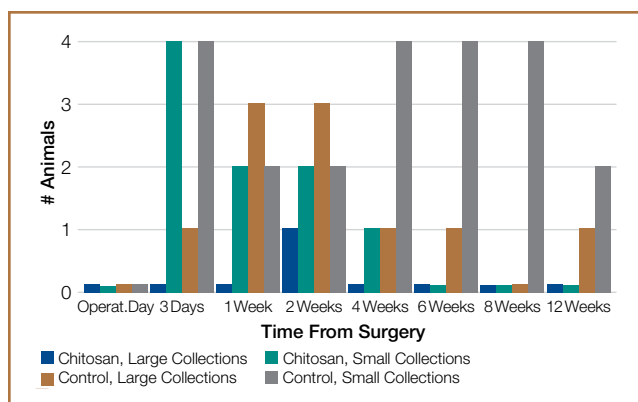


Figure 4. Distribution of inflammatory collections over time: numbers of rats with small and large collections. “Operat. day” refers to sacrifice 2 hours after surgery.

Table. Absence of Fatty Atrophy Over Time

| Time After Surgery | No. of Rats Without Fatty Atrophy | |
|----------------------------|-----------------------------------|---------|
| | Chitosan-Treated | Control |
| Operation day ^a | 5 | 5 |
| 3 days | 5 | 5 |
| 1 week | 5 | 5 |
| 2 weeks | 5 | 5 |
| 4 weeks | 4 | 3 |
| 6 weeks | 5 | 1 |
| 8 weeks | 4 | 0 |
| 12 weeks | 5 | 0 |

^aSacrifice 2 hours after surgery.

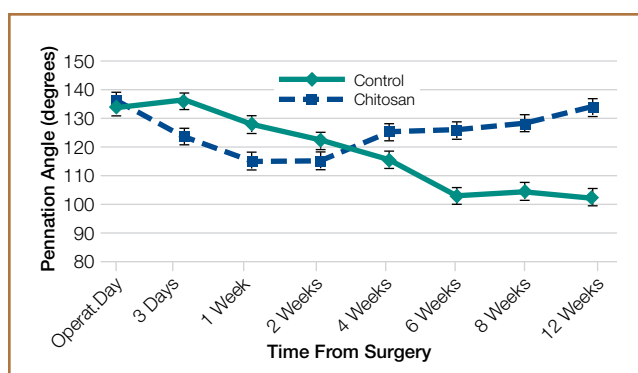


Figure 5. Pennation angle changes over time: means and standard deviations at each time point. “Operat. day” refers to sacrifice 2 hours after surgery.

operative rehabilitation.^{12,13} Repair strategies that biologically enhance the patient's intrinsic healing potential are needed.

In tendon repair, choice of repair material (eg, graft) is crucial in determining the success of tissue engineering approaches. The ideal scaffold is biocompatible and does not elicit a host inflammatory response. The selected scaffold in its composition and fabricated form must be capable of holding and supporting cells. In addition, the scaffold should be biodegradable, serving as a temporary support for such cells and mechanically augmenting the repaired tendon while allowing for eventual replacement by matrix components. Moreover, the scaffold should have high porosity and a large surface area. Furthermore, the material should mimic the native tendon extracellular matrix (ECM) architecture to allow cells to be distributed throughout the scaffold and to facilitate diffusion of nutrients and factors that promote cellular proliferation and ECM production.

Given the importance of glycosaminoglycans (GAGs) in supporting the reticular structure of the matrix, use of GAGs or GAG-analogues as components of a tendon tissue scaffold for enhancing repair is well documented.¹⁴ One such candidate is chitosan, a partially de-acetylated derivative of chitin found in arthropod exoskeletons. Structurally, chitosan shares some characteristics with various GAGs and hyaluronic acid.¹⁵ More specifically, chitosan is a linear polysaccharide composed of glucosamine and N-acetyl glucosamine units linked by β -glycosidic bonds. Investigators have studied the properties of chitosan, including its biocompatibility, biodegradability, antibacterial activity, mucoadhesivity, and wound healing.^{16,17}

One of the most promising features of chitosan is that it can be processed into porous structures for use in cell transplantation and tissue regeneration.^{18,19} Porous chitosan structures can be formed by freezing and lyophilizing chitosan-acetic acid solutions; chondrogenic cell adhesion and proliferation onto these structures have been reported.^{20,21} This chitosan scaffolding method has also been used to test different composites with collagens, gelatins, GAGs, and hyaluronic acid, all of which have also been proposed as useful 3-dimensional materials for tissue repair.²²

In the present study, we used chitosan matrix in RCT repair. We hypothesized that chitosan matrix could enhance rotator cuff repair the same way it enhances repair in epidermal tissues.¹⁶ Histologic findings demonstrated that the percentage of fibrous tissue was significantly higher in the chitosan-treated group than in the control group. This improved fibroblastic response may be attributed to the ability of chitosan to enhance cell migration and serve as a scaffold for repair. Other studies have indicated that chitin, of which chitosan is the primary derivative, accelerated the healing of skin and subcutaneous tissues by increased cell migration.²³ Moreover, Okamoto and colleagues²⁴ reported that chitin implants stimulated abundant angiogenesis through the same mechanism.

Inadequate initial strength of a repair may lead to a recurrent cuff tear or a disability of rotator cuff function in the early healing stages. In our study, the chitosan matrix tended to be absorbed by 6 weeks after surgery. Its adherence to and

ultimate absorption at the repair site may be challenged by the flow of irrigation fluid through the subacromial space in the setting of arthroscopic surgery. However, because the chitosan remains in a more robust gel form, it is better able to resist being washed from the repair site. For augmentation, it may be possible to apply a biocompatible patch over the gel to further protect it from being dislodged. In addition, histologic findings showed that the fibrous repair tissue gradually increased until reaching a peak 8 weeks after surgery—an indication that the absorption rate of the chitosan scaffold lags behind full recovery of the repair tissue. Given this relationship, further studies are needed to determine the mechanical strength of the repair between 6 and 8 weeks, which is important for avoiding recurrent tears.

Histologic findings
demonstrated that
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than in the control group.

This study had a few limitations. First, as with any animal model, the anatomy and function of the rat shoulder differ from those of the human shoulder. The acromial arch differs in quadruped animals, with less coverage of the supraspinatus and more of the subscapularis.²⁵ These anatomical differences could yield altered stress mechanics that could affect tendon repair. Furthermore, rats and humans differ in their RCT healing rates. Thus, the pathophysiology of muscle atrophy and fat infiltration in rats may slightly differ from that in humans. In addition, no mechanical testing was performed to compare chitosan-treated and untreated rotator cuff repairs, and such testing is needed to clarify the biomechanical importance of augmentation. Furthermore, no immunohistochemical analysis was performed for collagen. In the repair of rotator cuff tendons, surgeons must consider not only the number of cells but also the production of ECM. Although not directly confirmed in this study, chitosan induced fibrous tissue proliferation that mirrored production of a large amount of collagen fibers. Last, we used an open RTC model. As an arthroscopic model was not used, no definitive conclusions can be drawn regarding use of chitosan in arthroscopy.

Conclusion

Use of chitosan as an acellular matrix improved formation of healing fibrous tissue, increased the number of cells, and prevented fatty atrophy and inflammatory aggregates inside repair sites while facilitating recovery of the natural pennation angle of the tissue. These results demonstrate that chitosan can

enhance tendon healing in the setting of acute RCT. Further research, including biomechanical testing of repaired tendons, is needed to further delineate the utility of chitosan in regenerating irreparable RCTs.

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This paper will be judged for the Resident Writer's Award.
