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Fiber-aligned polymer scaffolds for rotator cuff repair in a rat model

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Background: Repair techniques of rotator cuff tendon tears have improved in recent years; nonetheless, the failure rate remains high. Despite the availability of various graft materials for repair augmentation, there has yet to be a biomechanical study using fiber-aligned scaffolds in vivo. The objective of this study was to evaluate the efficacy of fiber-aligned nanofibrous polymer scaffolds as a potential treatment-delivery vehicle in a rat rotator cuff injury model.

Materials and methods: Scaffolds with and without sacrificial fibers were fabricated via electrospinning and implanted to augment supraspinatus repair in rats. Repairs without scaffold augmentation were also performed to serve as controls. Rats were sacrificed at 4 and 8 weeks postoperatively, and repairs were evaluated histologically and biomechanically.

Results: Both scaffold formulations remained in place, with more noticeable cellular infiltration and colonization at 4 and 8 weeks after injury and repair for scaffolds lacking sacrificial fibers. Specimens with scaffolds were larger in cross-sectional area compared with controls. Biomechanical testing revealed no significant differences in structural properties between the groups. Some apparent material properties were significantly reduced in the scaffold groups. These reductions were due to increases in cross-sectional area, most likely caused by the extra thickness of the implanted scaffold material. No differences were observed between the 2 scaffold groups.

Conclusions: No adverse effect of surgical implantation of overlaid fiber-aligned scaffolds on structural properties of supraspinatus tendons in rat rotator cuff repair was demonstrated, validating this model as a platform for targeted delivery.

Level of evidence: Review Article.

Keywords: Rotator cuff; scaffold; tendon; biomechanics; injury; animal model; tissue engineering; shoulder

In the United States, musculoskeletal disorders have an annual economic impact of nearly $850 billion and affect more than 100 million adults.[1] Tears of the rotator cuff tendons represent a major component of the family of tendon disorders, affecting nearly half of the population aged older than 50 years.[22] An estimated

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75,000 rotator cuff repairs are performed each year, and techniques have improved substantially with time.[21] Nevertheless, failure rates of these repairs remain unacceptably high,[6] partly due to the limited ability for this tissue to heal and the nature of the disorganized tissue that is produced during the tendon-healing process. This inferior repair tissue and subsequent poor healing outcomes have brought about the need for a method to enhance regenerative healing.

One avenue for enhancement of regenerative healing is through the use of tissue engineering for direct tendon replacement,[7,16,17] for repair augmentation through mechanical bolstering of the healing tissue, or through cell and drug delivery. Recently, various graft materials and tissue-engineered constructs have been investigated to augment the repair.[4,8,11-13,15,19,23] Two recent investigations showed improvements or no detrimental effects on mechanical properties using nonaligned polymer scaffolds[8,19] and heparin/fibrin-based systems.[11]

A limited number of studies, however, have used fiber-aligned scaffolds, which mimic the alignment of collagen fibers in tendon. The architecture of tendon is made up of parallel collagen fibers that create an anisotropic tissue that is strongest along the tendon's long axis. Creating tissue-engineered constructs with aligned fibers similar to that of native tendon enables healing tissue to be directed and formed into this optimal orientation. Biphasic, aligned scaffolds have been used in an attempt to mimic the transitional nature of the tendon-to-bone interface in which tendon transitions into fibrocartilage, followed by mineralized fibrocartilage and eventually bone.[20] These scaffolds have shown promise as assessed by mineral deposition and cell proliferation and viability[12]; however, the use of fiber-aligned scaffolds in an in vivo animal model system has yet to be subjected to a rigorous biomechanical evaluation.

Enhancement of cellular infiltration and distribution can also be achieved through the use of sacrificial content. Using soluble fibers to act as place holders, eliminated sacrificial fibers have been shown to increase cellular infiltration in aligned and nonaligned electrospun nanofibrous scaffolds.[2,3,18] In addition to supporting repair through directed tissue deposition and increased cellular infiltration, an aligned scaffold for rotator cuff repair that uses sacrificial content could provide a platform for targeted delivery of factors to achieve the needed improvement in the healing process.

The objective of this study was to evaluate the biomechanical efficacy of 2 fiber-aligned polymer scaffolds as potential treatment delivery vehicles in a rat rotator cuff injury model. We hypothesized that the scaffold would not impair healing of the injury, as assessed through quantitative biomechanical testing and qualitative histologic assessment, and thus could serve as a platform for targeted delivery of healing factors.

Materials and methods

Fiber-aligned nanofibrous scaffolds were fabricated using a custom electrospinning setup.[2,10] Quantitative assessment of fiber alignment using the fabrication settings used in this work has shown that fibers are >90% aligned within ±20° of the prevailing fiber direction.[10] Briefly, a poly(ε-caprolactone) (PCL) solution was prepared and delivered along a high-voltage gradient onto an electrically grounded rotating mandrel, where it collected to form a pure PCL fiber-aligned mesh.

In a second spinning process, the PCL solution was co-electrospun with water-soluble poly(ethylene oxide) (PEO) solution. PEO acts as a sacrificial fiber when submersed in 90% ethanol, followed by distilled water, allowing for the creation of aligned PCL scaffolds with lower fiber density while maintaining fiber alignment. This co-electrospinning process was used for the formation of a second fiber-aligned mesh consisting of 60% PCL and 40% PEO, as determined by comparison of dry weight before and after submersion.[2] Individual implantable scaffolds of each type were cut to 3 mm wide × 5 mm long in the direction of fiber alignment. Scaffolds were sterilized and stored in sterile water at room temperature until surgery.

Forty male Sprague-Dawley rats (obtained at 400-450 grams) underwent a bilateral supraspinatus detachment and repair surgery. Shoulders were randomly assigned to have no scaffold (control; n = 24), a pure PCL scaffold (PCL; n = 28), or a dual-polymer scaffold (PCL/PEO; n = 28) to augment the repair.

Briefly, scaffolds were preloaded with suture using a grasping modified Mason-Allen stitch in preparation for implantation (Fig. 1). After supraspinatus tendon exposure and detachment, as we have described in detail previously,[20] scaffolds were implanted using a simple overlay by suturing along the anterior and posterior borders of the scaffold and supraspinatus tendon in a running Krakow fashion (Fig. 1). The supraspinatus tendon was repaired back to the greater tuberosity using a modified Mason-Allen technique.[20] Rats were allowed normal cage activity and were euthanized at 4 and 8 weeks after injury and repair (n = 12-14 shoulders per group).

For histologic assessment of the scaffold, 2 supraspinatus tendons per time point were grossly harvested from the shoulder, leaving the muscle and bony insertions intact. Specimens were fixed, decalcified, processed, and paraffin-embedded using standard techniques. Coronal sections were cut at 7-μm thickness and stained with hematoxylin and eosin. Slides were evaluated for identification of scaffold and qualitatively assessed for general levels of cellular infiltration and colonization.

For biomechanical testing, 12 supraspinatus tendons per group were dissected free from all muscle and extraneous connective tissue, while leaving the bony insertion to the proximal humerus intact. Verhoeff's stain lines were placed on the tendon at multiple locations for measurement of optical strain. Tendon cross-sectional area was measured using a custom device equipped with translational stages, 2 orthogonal linear variable differential transformers to measure position, and a charge-coupled device laser to measure specimen thickness.[5] This value was recorded and used for comparisons of specimen geometry between groups as well as in the calculation of material properties. After area measurement, the tendon end was fixed between 2 layers of sandpaper using a cyanoacrylate adhesive and clamped using

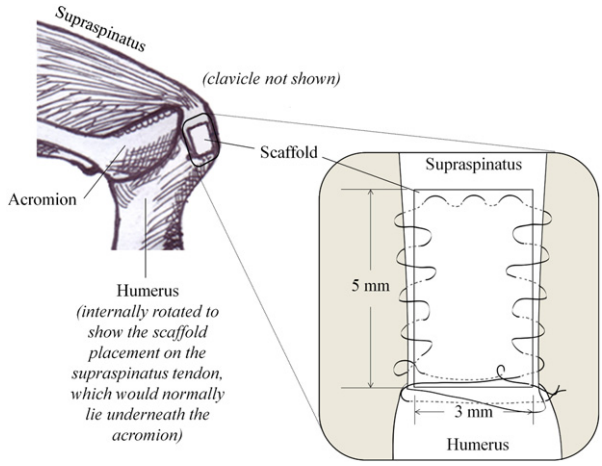

Figure 1 Schematic shows surgical placement of the implanted scaffold in an overlay fashion. For purposes of illustration, the clavicle is not shown and the humerus is shown internally rotated to allow visualization of the supraspinatus tendon and scaffold, which would normally lie underneath the acromion.

custom grips. The remaining portion of the humeral diaphysis was potted in polymethylmethacrylate and placed in a base fixture.

Specimens were submerged in a 37°C phosphate-buffered saline bath and tensile tested using an Instron 5543 mechanical test frame (Instron Corp, Norwood, MA, USA) using a protocol described previously.[14] Briefly, after a manual preload to eliminate slack, tendons were subjected to 10 cycles of preconditioning between and 0.1 and 0.5 N to obtain a consistent strain history between specimens. After returning to equilibrium over 300 seconds, a stress relaxation ramp to 5% strain was performed at a rate of 5% per second, followed by a 600-second relaxation. Tendons were then returned to their initial gauge length for 60 seconds and failed in tension at 0.3% per second.

Parameters evaluated included viscoelastic peak and equilibrium load/stress, percentage of relaxation, maximum load/stress, stiffness, and elastic modulus. Data between groups were compared at each time point using analysis of variance, followed by the Tukey post hoc test for multiple comparisons between individual groups. Statistical significance was set at $P \leq .05$.

Results

Examination of histology revealed that the implanted scaffolds remained in situ without gross migration from the supraspinatus tendon during the study interval; however, scaffold attachment to the bone appeared to not be maintained. The PCL scaffolds also showed appreciable cellular infiltration and colonization at 4 and 8 weeks after injury and repair (Fig. 2, *A-D*). Cells had colonized markedly around the edges and were also qualitatively more densely populated on the side closest to the site of injury. Visually, the PCL/PEO scaffolds showed noticeably less cellular infiltration at either time point (Fig. 2, *E-H*).

Dissected specimens from both scaffold groups were significantly larger in cross-sectional area compared with controls at 8 weeks after injury and repair ($P < .0001$). In particular, PCL specimens were larger than controls at 8 weeks ($P < .0001$) in addition to PCL/PEO being larger ($P < .0001$) at 8 weeks.

Biomechanical testing revealed no significant differences in structural properties (ie, maximum load, stiffness) between groups (Table I). Findings in apparent material properties were consistent with changes in the cross-sectional area. Reductions in peak ($P = .004$) and equilibrium ($P = .008$) stresses at 8 weeks were noted in the PCL group compared with controls. The PCL/PEO group also resulted in decreased peak ($P = .009$) and equilibrium ($P = .04$) stresses at 8 weeks. No differences were seen in elastic modulus or percent relaxation. Further, no differences were observed between PCL and PCL/PEO groups in any parameter at 4 or 8 weeks.

Discussion

Healing mechanical properties were assessed in rat supraspinatus tendon repairs augmented with 1 of 2 different fiber-aligned overlaid PCL-based scaffolds. As hypothesized, both scaffold groups showed no loss of structural properties compared with nonscaffold controls; however, reductions in stress were observed in the scaffold groups at 8 weeks. Importantly, these reductions in apparent material properties are due to calculations resulting from increases in cross-sectional area, most likely caused by the extra thickness (0.83 ± 0.08 mm for PCL and 0.79 ± 0.10 mm for PCL/PEO) of the implanted scaffold material and not due to an increased fibrotic or scar response in these groups. We therefore conclude that the PCL and PCL/PEO scaffolds did not have a detrimental effect on the structural mechanics of the healing supraspinatus tendons in this model system.

Qualitative histologic examination showed that the PCL scaffolds were well infiltrated and colonized with cells at 4 and 8 weeks after injury and repair, although this finding was less apparent in the PCL/PEO group despite their higher sacrificial content. This result was surprising based on previous work from our laboratory that showed increased cellular infiltration in scaffolds with sacrificial content after in vitro cell culture[2] and in vivo subcutaneous implantation.[3] This discrepancy may be due to the handling of the scaffolds before implantation. With 40% of the fibers being removed by design in the PCL/PEO group, it is possible that the scaffolds were more easily compressed during the presuturing component of the surgical procedure or by the nature of the anatomic location in which they were placed. This compression would have led to the elimination of the spaces vacated by the sacrificial fibers, leading to less space overall available for colonization.

Previous studies have reported the use of various scaffolds in rat rotator cuff models. Nonaligned scaffolds and

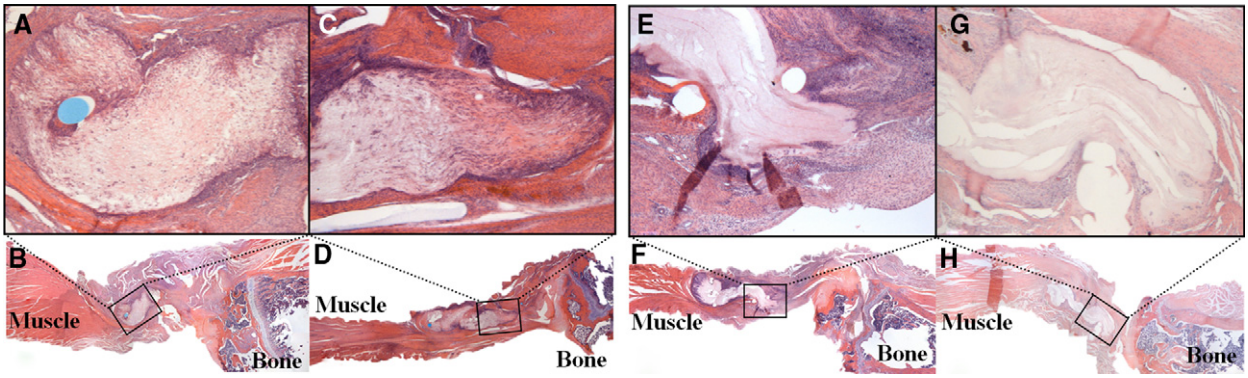

Figure 2 Photomicrographs (original magnification ×50) and panoramic views of hematoxylin and eosin stained specimens from the **(A, B)** poly(ε-caprolactone) (PCL) group at 4 weeks and **(C, D)** 8 weeks after injury and repair show PCL scaffold cellular infiltration and colonization. PCL/poly(ethylene oxide) (PEO) specimens were not well-infiltrated at **(E, F)** 4 or **(G, H)** 8 weeks. Slides were stained together in a single batch and qualitatively and visually assessed for cellularity. Apparent variations in staining intensity were not evaluated between groups.

Table I Cross-sectional area and mechanical testing results at 4 and 8 weeks*

Group	Area (mm^2)	Max load (N)	Max stress (MPa)	Stiffness (N/mm)	Modulus (MPa)
4 weeks					
Control	7.19 ± 1.79	10.8 ± 4.61	1.56 ± 0.68	21.2 ± 13.3	15.5 ± 9.0
PCL	9.22 ± 2.74	9.01 ± 3.38	1.03 ± 0.40	17.3 ± 8.3	10.3 ± 8.0
PCL/PEO	8.83 ± 1.73	10.8 ± 4.40	1.28 ± 0.57	20.4 ± 8.6	11.4 ± 6.8
8 weeks					
Control	6.52 ± 1.08	15.3 ± 7.25	2.33 ± 1.07	31.9 ± 14.3	21.7 ± 12.0
PCL	10.3 ± 1.77†	18.0 ± 8.99	1.82 ± 0.91	31.4 ± 12.3	15.3 ± 8.2
PCL/PEO	9.87 ± 1.47†	16.8 ± 5.43	1.69 ± 0.46	34.1 ± 13.9	15.6 ± 6.0

* Data are presented as means ± standard deviation. Structural properties were not inhibited by the addition of poly(ε-caprolactone) (*PCL*) or PCL/poly(ethylene oxide) (*PEO*) scaffolds.
† Significant differences compared with control group.

matrices have been used and have shown improvements in mechanical properties:

- One study used nonaligned poly(85 lactic acid-co-15 glycolic acid) (PLAGA) scaffolds in an overlay fashion to augment rat supraspinatus detachment and repair.[19] Results showed a dramatic increase in elastic modulus at 8 weeks with the scaffold compared with no scaffold repairs. No difference was seen at 4 weeks between groups.
- Another study used a nonaligned heparin/fibrin-based gel as an overlay to deliver transforming growth factor-β3 (TGF-β3) to rat supraspinatus injury and repair.[11] Inclusion of TGF-β3 in the scaffold improved mechanical properties, but there was no change when using scaffolds without TGF-β3 compared with repair-only controls.
- Porcine small intestine submucosa has been used to augment repair in multiple investigations, with results showing no change compared with repair-only controls as well as improvements in mechanical properties over time after repair and implantation.[13,15,23]

The overall trends of these studies are consistent with the present findings of no detrimental changes in structural properties caused by scaffold implantation. Our study, however, represents the first in vivo implementation of electrospun, fiber-aligned scaffolds, followed by biomechanical and histologic evaluation.

Conclusion

No adverse effect of surgical implantation of overlaid fiber-aligned scaffolds on structural properties of supraspinatus tendons in rat rotator cuff repair was demonstrated, supporting our hypothesis and validating this model as a platform for targeted delivery. Although both types of PCL scaffold used in the current study were receptive to cellular infiltration, future work will focus on the co-electrospun PCL/PEO scaffolds. With improved handling techniques, the elimination of the PEO component will allow for enhanced cell colonization and subsequent drug delivery, as well as the addition

of cells and other factors to enhance the healing capacity of the repair.

Future studies may also use polymeric microspheres[9] embedded within the sacrificial fibers of PCL/PEO scaffolds to enable drug delivery in this model. More specifically, future work will deliver nonsteroidal anti-inflammatory drugs to investigate their influence from local (via scaffold) vs systemic (eg, oral) application, as well as determining the effect of dose response of nonsteroidal anti-inflammatory drugs early in the healing process of this model. Additional studies may also assess the effects of other cells and factors such as interleukin-1 receptor antagonist, platelet-derived growth factor, vascular endothelial growth factor, and TGF. Augmentation of the repair combined with concurrent drug delivery in this manner could enhance long-term healing, the limit apoptosis and inflammation in the short-term after rotator cuff injury and repair, and might eventually lead to improvement of clinical surgical outcome by enhancing tissue regeneration.

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