Published in final edited form as:

Tech Orthop. 2016 June ; 31(2): 91–97. doi:10.1097/BTO.0000000000000168.

Current Status of Tissue-Engineered Scaffolds for Rotator Cuff Repair

Abby Chainani, B.S. and Dianne Little, D.V.M, Ph.D.

Department of Orthopaedic Surgery, Duke University Medical Center, Durham, NC 27710

Abstract

Rotator cuff tears continue to be at significant risk for re-tear or for failure to heal after surgical repair despite the use of a variety of surgical techniques and augmentation devices. Therefore, there is a need for functionalized scaffold strategies to provide sustained mechanical augmentation during the critical first 12-weeks following repair, and to enhance the healing potential of the repaired tendon and tendon-bone interface. Tissue engineered approaches that combine the use of scaffolds, cells, and bioactive molecules towards promising new solutions for rotator cuff repair are reviewed. The ideal scaffold should have adequate initial mechanical properties, be slowly degrading or non-degradable, have non-toxic degradation products, enhance cell growth, infiltration and differentiation, promote regeneration of the tendon-bone interface, be biocompatible and have excellent suture retention and handling properties. Scaffolds that closely match the inhomogeneity and non-linearity of the native rotator cuff may significantly advance the field. While substantial pre-clinical work remains to be done, continued progress in overcoming current tissue engineering challenges should allow for successful clinical translation.

Keywords

Electrospinning; interfacial; stem cell; nanofiber; microfiber

Introduction

The prevalence of rotator cuff tear increases with age to over 50% in adults over the age of 60,^{1, 2} and more than 300,000 rotator cuff repair surgeries are performed annually in the United States.³ Re-tear rates may be high,⁴ and factors associated with high failure rates include chronicity,⁵ tear size,⁵ fatty atrophy of rotator cuff muscles,⁶ tendon retraction or failure with continuity,⁷ failure to regenerate the normal tendon-bone interface,⁸ poor tissue quality, surgical technique, and postoperative rehabilitation protocols.⁹ The use of allograft, autograft, and xenografts for rotator cuff reconstruction and augmentation is described by Gupta and Toth in Chapter XXX. However, a recent consensus statement from the American Academy of Orthopedic Surgeons was unable to recommend for or against the use of soft tissue allografts or other xenografts for treatment of rotator cuff repairs due to insufficient or

*Address correspondence to: Dr. Dianne Little, Orthopaedic Research Laboratories, DUMC Box 3093, 375 MSRB1, Durham, NC 27710, USA. Tel.: 1 (919) 681-6867; Fax: 1 (919) 681-8490. dianne.little@duke.edu.

Conflicts of Interest: Dr. Little is a paid consultant for Cytex Therapeutics Inc. and received an unrestricted post-doctoral fellowship from Synthes USA in support of part of this work. For the remaining authors none were declared.

conflicting evidence.¹⁰ These challenges have motivated investigation into development of alternative constructs that can stimulate rapid tendon regeneration and provide mechanical augmentation, ideally within the first 12 weeks post-operatively.⁷ An further unmet need for engineered scaffold development is in the area of interpositional devices for irreparable tears, since none of the currently available devices have been cleared by the FDA for this application.¹¹ However, any device in the future that is successful as an interpositional device is also be likely to be useful for augmentation repair of massive tears, and in achieving a functional tendon-bone interface. This review will focus on recent basic science and pre-clinical approaches to engineered tissue engineered scaffolds for rotator cuff repair.

Desirable Attributes of Tissue Engineered Scaffolds

Functional tissue engineering of the rotator cuff combines various scaffolds (porous meshes, woven and non-woven fibers, composites of different biomaterials and hydrogels), cells, bioactive molecules (growth factors, transcription factors and proteins to induce differentiation, proliferation, and metabolic activity of cells), and in some cases the use of a bioreactor¹² to achieve physiological tendon, muscle-tendon, and tendon-bone function and mechanical properties. The specific requirements for physiological functioning of a tissue engineered tendon are almost as complex as the native rotator cuff, but in general, scaffolds should be:

- 1) Cell-instructive for both tendon and the tendon-fibrocartilage-bone interface by providing micro-architectural and bioactive cues for cellular differentiation, proliferation and maintenance of tissue-specific matrix and cellular activity.
- 2) Intact with adequate mechanical properties to support the repair for at least 12 weeks after implantation¹³ and mimic the loading environment of the native tissue in order to provide mechanoactive cues for cellular differentiation.¹⁴
- 3) Slowly degradable to enable the new tendon and tendon-bone interface to fully integrate and regenerate. Non-toxic degradation by-products should be readily metabolized or cleared from the body without generating a foreign body response.^{15, 16}
- 4) Porous to permit cell infiltration, nutrient exchange and angiogenesis, and have good suture retention and surgical handling characteristics.
- 5) Resistant to wear and infection, and be biocompatible without causing inflammation or immune reactions in a synovial environment.
- 6) Amenable to arthroscopic or mini-open approaches in which the thickness of both the initial scaffold and the remodeled engineered tendon is restricted to reduce likelihood of sub-acromial impingement.¹⁷
- 7) Stable to sterilization, processing and storage conditions.
- 8) Able to demonstrate an improved risk:benefit ratio compared to the current standard of care.

These attributes have been evaluated to different extents in a variety of biodegradable foam, woven and non-woven fiber, and composite scaffolds. Development of tissue engineered scaffolds is still largely in *in vitro* static culture and bioreactor phases with some early pre-clinical *in vivo* studies emerging.

Current *in vitro* work is focused on developing appropriate mechanical properties, degradation profiles, and cell-instructive scaffolds. A variety of scaffolds have been produced using different techniques and polymers, and cultured with several different cell types, using a number of different strategies to enhance the bioactivity of scaffolds. Relatively few scaffolds have moved to small animal studies, and even fewer have been evaluated in large animal models. The remainder of this review will focus on recent work in each of these areas.

Mechanical properties and degradation profile

The critical time frame for mechanical augmentation is in the first 12 weeks post-operatively.^{7, 13} As a minimum, to achieve adequate mechanical support immediately after repair, scaffolds should aim to approximate the stiffness (~200 N/mm), modulus (~150MPa) and ultimate load (~800 N) of human rotator cuff combined with adequate suture retention properties.^{16, 18} However, the complex regional inhomogeneity and non-linearity of mechanical properties and structure of the supraspinatus tendon,^{18–20} and multiaxial loading environment of the rotator cuff suggest that a more nuanced approach to scaffold design may be necessary for long term success. This approach would improve matching of regional scaffold properties to that of the native tendon, and matching of the complex interactions between different layers of the rotator cuff that result in differential strain between joint and bursal sides of the tendons of approximately 10%^{18, 19, 21}.

Current work in polymer selection and scaffold fabrication aims to achieve adequate initial mechanical properties and desired degradation kinetics, increase rate of tissue formation and maturation to mitigate loss of mechanical properties caused by degradation, manipulate scaffold mechanical anisotropy, and attempt to recapitulate some of the intricate anatomical features in the anterior-posterior, lateral-medial and bursal-synovial planes of the rotator cuff, and its tendon-bone interface. Achieving adequate initial mechanical support coupled with a slowly degrading scaffold that permits complete tissue integration and replacement is challenging, since degradation kinetics of a scaffold are determined by properties of the polymer, biocompatibility, host response, structural properties (eg. fiber diameter, fiber alignment, porosity, fabrication method), and processing and sterilization techniques.^{22–24}

Polymer Selection for Rotator Cuff Tendon Tissue Engineering

Many of the polymers currently under investigation for rotator cuff tendon tissue engineering are already FDA-approved for other applications. Both poly(glycolic acid) PGA and poly(lactic acid) (PLA) have adequate initial elastic moduli for rotator cuff tissue engineering but PGA degrades too rapidly (on the order of several weeks) to be useful by itself for rotator cuff applications. Compared to PGA, PLA degrades more slowly (on the order of many months), but is more hydrophobic. Copolymers of PLA and PGA, such as

poly[DL- lactic-co-glycolic acid] (PLGA) are the most frequently used applications of PLA and PGA in rotator cuff tendon tissue engineering since PLGA blends have excellent biocompatibility and tunable mechanical and degradation properties. A major concern regarding the use of these polymers in rotator cuff tissue engineering applications is the acidic intermediate degradation products, lactic acid and glycolic acid. These degradation products reduce the pH at the site of implantation, and induce an inflammatory response. This is of particular concern at the tendon-bone interface, where the potential for osteolysis and failure of the repair is high.²⁵ To buffer these pH changes, additives such as hydroxyapatite or bioactive glass have been incorporated into the scaffolds and are themselves osteo-conductive and osteo-inductive.^{26, 27} Poly (ϵ -caprolactone) (PCL) generally has a lower elastic modulus than PGA, PLA or PLGA blends,²⁸ but strain at failure is greater, and degradation kinetics are slower than PGA, PLA or PLGA blends. Recently ultra-high molecular weight PCL has been reported to be comparable to anterior cruciate ligament allografts in the rat,²⁹ and this polymer may prove to be useful for rotator cuff tendon tissue engineering. While 6-hydroxylcaproic acid is produced as an intermediate of hydrolysis during degradation of PCL, no osteolysis has been identified in long term implantation studies in animal models, and PCL is currently one of the most widely used polymers in the field of tissue engineering.³⁰ Other polymers are undergoing evaluation and modification for tissue engineering applications and may be evaluated in the future for rotator cuff tendon tissue engineering. For example, the polyurethanes have excellent tensile mechanical properties for potential rotator cuff applications, but are generally non-absorbable. Recent work to incorporate biodegradable components into the segmental chemistry of polyurethanes and allow alternate degradation kinetics,³¹ represents an exciting area of future investigation. Poly(desaminotyrosyl-tyrosine dodecyl dodecanedioate) (p(DTD-DD))-derived, tyrosine-based novel polymers are also potential candidates for rotator cuff tendon tissue engineering, and have enhanced mechanical properties compared to PCL.³² The functional tyrosine groups can be modified to enhance the biomimetic potential of these polymers and allow for tunable degradation properties.³³

Proteins such as collagen, hyaluronic acid, and silk³⁴ have also been used to fabricate scaffolds but typically have inferior mechanical properties compared to synthetic polymers. More complex extracellular matrices such as tendon-derived matrix have also been investigated for rotator cuff tissue engineering, either by coating onto the surface of polymer fibers,³⁵ or by incorporating the matrix directly into the polymer fiber.³⁶ Several challenges associated with incorporation of proteins into scaffolds include maintaining the biomimetic activity of the proteins, and retaining them within the scaffold.

Scaffold Fabrication for Rotator Cuff Tendon Tissue Engineering

A variety of woven, non-woven, foam-based, tissue-derived and composite scaffolds are under investigation for rotator cuff tendon tissue engineering. Several of the woven and non-woven scaffolds are limited to microscale design features, but increasingly attempts are being made to recapitulate the nanoscale architecture of collagen fibers within the tendon or at the tendon-bone interface.³⁷ A variety of methods are used to produce tissue-engineered nanofibers including self-assembly, drawing temperature-induced phase separation, and perhaps most commonly electrospinning.^{28, 37} Electrospinning is as simple and readily

controllable process^{37, 38} that generates non-woven nanofibers by application of high voltage to a polymer solution as it is extruded from a needle. As jets of the charged solution accelerate towards a grounded collector, the solvent evaporates leaving fibers of 500–1500nm that are deposited on the collector as a non-aligned non-woven nanofiber scaffold.

Nanofiber organization is a critical component for scaffold design, particularly at the tendon-bone interface.^{24, 39} Aligned scaffolds that closely mimic the aligned collagen fiber architecture and anisotropic mechanical properties of tendon can readily be fabricated by using different configurations of the grounded collector.^{40–54}

Major limitations of electrospinning for rotator cuff tendon tissue engineering have been achieving adequate mechanical properties and porosity to permit complete cell infiltration.⁵⁵ Recently, several groups have made progress to increase porosity and increase cell infiltration and extracellular matrix synthesis through the full thickness of scaffolds by developing multilayered scaffolds with a variety of modifications for collection of the electrospun fibers.^{35, 56–64} Clear patterns of extracellular matrix organization in response to micro-architectural cues provided by the scaffolds are emerging, with multi-layered scaffolds containing only non-aligned fibers demonstrated cell infiltration and new type I and III collagen synthesis through the full thickness of the scaffolds, whereas scaffolds containing only aligned fibers additionally demonstrated organized and aligned collagen fibers through the full thickness when evaluated by polarized light microscopy.⁴⁷

Together, the body of evidence from *in vitro* evaluation of nanofiber scaffolds suggests that potent cell instructive micro-architectural cues can be manipulated to enhance the rate of development of engineered tendon, and to provide differentiation cues towards not only tendon, but components of the tendon-bone interface, including un-mineralized and mineralized fibrocartilage. However, in order to reduce mismatch between the regional inhomogeneity and anisotropy of the rotator cuff tendons,^{18, 65} and to minimize stress concentration at the tendon-bone interface, it may also be necessary to recapitulate the complex multiaxial alignment patterns of different layers of the rotator cuff, through incorporating this regional anisotropy and these regional gradients into scaffold design.⁶⁶ Together these advances are likely to significantly improve the chances of successful translation of nanofiber technology to rotator cuff devices. The focus of the majority of current work is transitioning to pre-clinical studies and strategies to allow commercial scale-up of non-woven nanofiber technologies.

Cells and Differentiation Pathways

Two basic approaches are used when using cells in rotator cuff tendon tissue engineering: First, several groups have attempted to recapitulate the different cell lineages at the tendon-bone interface using different cell populations (osteoblasts, chondrocytes and fibroblasts) seeded onto scaffolds, however, this approach poses significant barriers to translation.⁶⁷ Second, a single cell type is differentiated towards a tenogenic lineage, or towards three different lineages (tenogenic, fibrochondrogenic and osteogenic) for regeneration of the tendon-bone interface. A variety of cell populations have been used for rotator cuff tendon tissue engineering: rotator cuff fibroblasts and tendon progenitor cell populations, periosteal

cells and mesenchymal stem cells (bone-marrow, adipose, sub-acromial bursa or umbilical cord derived). All of these cell sources exhibit the ability to maintain or undergo differentiation towards a tenocyte-like lineage, but experimental conditions are important. To our knowledge there have been no studies to compare the degree of tenogenic differentiation capacity by multiple different cell sources under the same *in vitro* or *in vivo* conditions. Studies to evaluate cell differentiation towards a tendon lineage and maturation towards a mature tendon cell are complicated by the current lack of specific molecular markers of tendon cell identity, therefore current approaches rely on a panel of markers such as transcription factors relating to tendon development, regeneration and differentiation (Scleraxis, Mohawk and Early Growth Response family members 1 & 2 (Egr 1 & Egr2), and extracellular matrix proteins and glycoproteins that are critical either for tendon differentiation, structure, or for regulation of tendon extracellular matrix (Type I and III Collagen, Tenascin C, Tenomodulin, Decorin, and Biglycan).⁶⁸ Another limitation in the field that is currently being addressed is incomplete understanding of the signaling pathways that regulate enthesis development; this limits the ability to successfully differentiate cells towards all of the cell lineages identified at the enthesis. However, hedgehog signaling has recently been found to be critical for enthesis development and maturation; continued work in this area will shed further light on enthesis formation at the rotator cuff.⁶⁹

Differentiation of cells towards a tendon lineage or to regeneration of the tendon-fibrocartilage-bone interface using biomimetic factors can be achieved through several approaches: 1) Direct delivery of growth factors or transcription factors to cells from the scaffold or extracellular environment. 2) Transduction or transfection of cells to produce growth factors or induce tenogenesis through a 'gene therapy' approach. 3) Use of complex extracellular matrices to supply endogenous levels of both growth factors and matrikines to stimulate differentiation. Growth factors are cytokines that promote cell division, maturation, and differentiation; whereas matrikines are peptides contained within extracellular matrix proteins that bind cell surface receptors to direct cell-matrix interactions and cellular differentiation. Use of these differentiation techniques is becoming increasingly sophisticated in order to both retain the biomimetic factors at the location of interest and to recapitulate the gradient of the tendon-bone interface within the scaffold used for repair.⁷⁰

1) Direct delivery of growth factors or transcription factors for rotator cuff repair

A variety of growth factors have been evaluated for their effect on tendon and tendon-bone healing, including several members of the bone morphogenetic protein (BMP) and transforming growth factor (TGF) families. In addition, basic fibroblast growth factor (FGF-2) has beneficial effects on healing including enhanced new bone formation and reduced delamination of the tendon-bone interface in sheep, but tendon thickness is increased, raising translational concerns for inducing sub-acromial impingement.⁷¹ However, in rats FGF-2 delivery enhanced mechanical properties without increasing cross-sectional area, and increased collagen organization and expression of scleraxis and tenomodulin in the healing interface.⁷² Direct delivery of platelet-derived growth factor-BB (PDGF-BB) improved collagen organization, and mechanical properties but not regeneration of fibrocartilage at the rotator cuff tendon-bone interface in rats.⁷³ Together, these studies suggest that while direct growth factor delivery may improve individual aspects of repair,

this technique is unlikely to improve all aspects of tendon or tendon-bone healing or regeneration.

2) Gene therapy for rotator cuff repair

Gene therapy provides a delivery method for sustained release of growth or transcription factors or other signaling proteins and depending on the method of delivery can be switched 'on' or 'off'. Transfection or transduction of various proteins have been evaluated for their effects on tendon differentiation and matrix synthesis including vascular endothelial growth factor (VEGF), growth differentiation factor-5 (GDF5), matrix metalloproteinase (MMP)-14, PDGF- BB, insulin-like growth factor-1 (IGF-1), FGF-2 and granulocyte-colony stimulating factor (G-CSF) and of these, IGF-1 appears to be one of the most promising growth factors for tendon healing.⁷⁴⁷⁰⁷⁵. Transduction of mesenchymal stem cells with the transcription factor scleraxis enhances tendon repair in rat models,⁷² while Egr1 over-expression in tendon stem cells enhances rotator cuff repair in rabbit models through BMP12/Smad1/5/8 signaling.⁷⁶ For engineering of the interface between tendon and bone, immobilization of a TGF- β 3 expressing lentiviral vector onto a scaffold induced a chondrogenic phenotype by mesenchymal stem cells.⁷⁷ Not all studies to evaluate the efficacy of growth factors are as successful when applied to animal models as *in vitro* studies would suggest. For example, adipose stem cells transduced with BMP-2 and seeded onto aligned PLGA scaffolds in an attempt to improve tendon-bone healing had *reduced* trabecular bone at the tendon-bone interface and reduced mechanical properties compared to scaffolds not producing BMP-2 in a rat model, therefore use of BMP-2 for enhanced tendon-bone repair at the rotator cuff was not recommended; these results were consistent with several similar studies previously reported, but also in contrast to other studies suggesting a positive effect of BMP-2 in tendon-bone or ligament-bone healing.²⁵ However, more complex use of growth factors such as opposing gradients of BMP-2 and PDGF-BB within a single scaffold may more closely recapitulate the native tendon-bone interface.⁷⁸ This latter example demonstrates the difficulties associated with use of growth factors for rotator cuff tendon tissue engineering. It is unlikely that one or even two individual growth factors will be able to induce and maintain the complex environment of the native rotator cuff and its insertion, where many growth factors, other proteins and regulatory elements act in a coordinated manner to achieve rotator cuff development and homeostasis.

3)Use of Complex Extracellular Matrices

The discovery 50 years ago by Marshall R. Urist M.D. of the osteoinductive and osteogenic properties of demineralized bone matrix has led to substantial interest in the use of demineralized bone and other naturally occurring extracellular matrices to enhance tissue repair. A substantial body of work now exists to demonstrate that extracellular matrix derived from cartilage, or tendon or ligament has the potential to enhance musculoskeletal soft tissue repair along their respective differentiation lineages [reviewed in ⁷⁹], possibly because of the complex array of growth factors, matrikines and other ligands that they contain. *In vitro* studies have confirmed that processed cartilage or tendon matrices incorporated into electrospun scaffolds have potent chondrogenic or tenogenic capacity, and can induce more rapid cellular infiltration and matrix synthesis and alignment through the full thickness of the scaffold (Figure 1) than would be expected from use of polymer

alone.^{35, 36, 80} As expected, the tensile mechanical properties of electrospun scaffolds containing only tendon-derived matrix were poor (unpublished data), but improved tensile properties were observed when tendon-derived matrix was blended with polymer.³⁶ The compressive modulus of electrospun scaffolds is inadequate to support mechanical load within a diarthrodial joint, particularly when blended with cartilage-derived matrix,⁸⁰ but the chondrogenic effects of cartilage-derived matrix incorporated into electrospun scaffolds may be beneficial when inserted between tendon and bone at the tendon-bone interface and is the subject of ongoing investigation. The method of preparation and incorporation of extracellular matrices such as tendon into scaffolds influences the biomimetic properties observed.⁶⁶ Therefore substantial work remains to determine the optimal method by which these native extracellular matrices can be incorporated into scaffolds to maximize their potential biomimetic effects in tendon and at the tendon-bone interface.

Host Response

Comprehensive evaluation of host response to a scaffold or biomaterial for rotator cuff repair should include consideration both of the pathological inflammatory responses associated with activation of a foreign body response [reviewed in ⁸¹], and from an immunological perspective to examine immunomodulation of innate, humoral and cell-mediated responses induced by the biomaterial.⁸² Host response is influenced by biomaterial composition and morphology, including fiber orientation in non-woven scaffolds.⁸³ The degree of decellularization and removal of any potential xenogeneic antigens for xenograft material is also important,^{22, 79} as are biologic factors such as exposure to synovial fluid, and mechanical factors at the implantation site.¹⁶ To detect potential problems with host response before clinical trials, appropriate pre-clinical model selection, design and outcome measure evaluation is necessary. In this regard the rat rotator cuff is relatively insensitive to mechanical load and synovial variables, and may be less valuable for predicting host response to biomaterials for rotator cuff repair than other species.⁸⁴ Further, consideration should be given as to whether the selected animal model will be capable of inducing a response to xenogeneic antigens.^{22, 79} Therefore, as pre-clinical studies are designed to evaluate candidate engineered scaffolds for rotator cuff repair, it is critical that host responses are fully examined in order to better predict biocompatibility, degradation, and resistance to infection.

Preclinical Studies

As alluded to in earlier sections, with all pre-clinical study design, careful selection of animal model, control groups and functional and end-point outcome measures is critical to allow valid assessment of the tissue engineered product. Accurate and careful reporting of methods allows comparison to be made between results from different studies. The ideal animal model for evaluation of scaffolds for rotator cuff tissue engineering has similar anatomy, function and size scale to human, an intra-synovial environment, ability to model chronic injury, re-tear and muscle atrophy, similar underlying ability to heal as human, the ability to incrementally rehabilitate the repair through loading and range of motion⁸⁵, and to assess clinically relevant functional outcome measures.⁸⁶ The most commonly used species used for translational models for the rotator cuff include the rat, rabbit, dog and sheep.⁸⁵

However, inherent limitations of the most commonly used animal models include quadrupedal gait pattern, difficulty in modulating limb loading following surgery, and a high, almost universal re-tear rate with failure to maintain tendon-bone contact. In this regard, several studies have evaluated several methods to unload limbs following rotator cuff tendon injury and repair,^{85–87} while other studies have evaluated novel methods of suturing engineered scaffolds within the repair interface.⁸⁸ Despite the limitations of these animal models, their high re-tear rates may in some respects represent a more stringent mechanical loading environment than humans, and any device capable of successfully reducing gap formation in the post-operative period in a large animal model may ultimately have adequate mechanical properties to improve functional in the post-operative period in humans. Relatively few tissue engineered devices have been evaluated in animals models of rotator cuff tear;⁸⁹ these studies have been predominantly in rat, where an overwhelming fibrovascular response to implanted PLGA scaffold was observed.²⁵ In contrast, an electrospun PCL scaffold implanted in a similar manner did not induce a profound fibrovascular response and was not detrimental to structural properties, but did not maintain attachment to bone.⁸⁹ Together these studies suggest that much work remains before successful translation. Once these substantial preclinical hurdles are cleared, controlled clinical trials are needed to investigate the human injury condition in order to determine the overall efficacy and safety of scaffold devices.¹⁶

Conclusion

The current body of basic research is significantly increasing the possibility that an engineered scaffold which provides for mechanical augmentation over the course of healing of the tendon and tendon-bone interface will be developed. While there is still a long way to go, there have been encouraging advances in the field and over time, teams of investigators will learn from each other's successes and failures, and from work in the fields of developmental and tendon biology and mechanobiology to meet the challenges that remain to be addressed.

Acknowledgements

The author's acknowledge the large body of work in this area that we were unable to cite due to space restrictions.

Funding for this work was received from the National Institutes of Health (AR059784 and AR055042) (DL), and an unrestricted post-doctoral fellowship from Synthes USA (DL).

References

1. Sher JS, Uribe JW, Posada A, et al. Abnormal findings on magnetic resonance images of asymptomatic shoulders. *J Bone Joint Surg Am.* 1995; 77:10–5. [PubMed: 7822341]
2. Yamaguchi K, Ditsios K, Middleton WD, et al. The demographic and morphological features of rotator cuff disease. A comparison of asymptomatic and symptomatic shoulders. *J Bone Joint Surg Am.* 2006; 88:1699–704. [PubMed: 16882890]
3. Colvin AC, Egorova N, Harrison AK, et al. National trends in rotator cuff repair. *J Bone Joint Surg Am.* 2012; 94:227–33. [PubMed: 22298054]
4. Neri BR, Chan KW, Kwon YW. Management of massive and irreparable rotator cuff tears. *J Shoulder Elbow Surg.* 2009; 18:808–18. [PubMed: 19487132]

5. Bartolozzi A, Andreychik D, Ahmad S. Determinants of outcome in the treatment of rotator cuff disease. *Clin Orthop Relat Res*. 1994;90–7. [PubMed: 7955708]
6. Goutallier D, Postel JM, Gleyze P, et al. Influence of cuff muscle fatty degeneration on anatomic and functional outcomes after simple suture of full-thickness tears. *J Shoulder Elbow Surg*. 2003; 12:550–4. [PubMed: 14671517]
7. McCarron JA, Derwin KA, Bey MJ, et al. Failure with continuity in rotator cuff repair “healing”. *Am J Sports Med*. 2013; 41:134–41. [PubMed: 23019253]
8. Rodeo SA. Biologic augmentation of rotator cuff tendon repair. *J Shoulder Elbow Surg*. 2007; 16:S191–7. [PubMed: 17574875]
9. Iannotti JP. Full-Thickness Rotator Cuff Tears: Factors Affecting Surgical Outcome. *J Am Acad Orthop Surg*. 1994; 2:87–95. [PubMed: 10708996]
10. National Guideline C. American Academy of Orthopaedic Surgeons clinical practice guideline on optimizing the management of rotator cuff problems. Agency for Healthcare Research and Quality (AHRQ); Rockville MD: 2010.
11. Derwin KA, Badylak SF, Steinmann SP, et al. Extracellular matrix scaffold devices for rotator cuff repair. *J Shoulder Elbow Surg*. 2010; 19:467–76. [PubMed: 20189415]
12. Youngstrom DW, Rajpar I, Kaplan DL, et al. A bioreactor system for in vitro tendon differentiation and tendon tissue engineering. *J Orthop Res*. 2015; 33:911–18. [PubMed: 25664422]
13. Kim JH, Hong IT, Ryu KJ, et al. Retear rate in the late postoperative period after arthroscopic rotator cuff repair. *Am J Sports Med*. 2014; 42:2606–13. [PubMed: 25186830]
14. Smith BD, Grande DA. The current state of scaffolds for musculoskeletal regenerative applications. *Nat Rev Rheumatol*. 2015; 11:213–22. [PubMed: 25776947]
15. Hakimi O, Mouthuy PA, Carr A. Synthetic and degradable patches: an emerging solution for rotator cuff repair. *Int J Exp Pathol*. 2013; 94:287–92. [PubMed: 23837794]
16. Ricchetti ET, Aurora A, Iannotti JP, et al. Scaffold devices for rotator cuff repair. *J Shoulder Elbow Surg*. 2012; 21:251–65. [PubMed: 22244069]
17. Cho CH, Lee SM, Lee YK, et al. Mini-Open Suture Bridge Repair with Porcine Dermal Patch Augmentation for Massive Rotator Cuff Tear: Surgical Technique and Preliminary Results. *Clin Orthop Surg*. 2014; 6:329–35. [PubMed: 25177460]
18. Lake SP, Miller KS, Elliott DM, et al. Effect of fiber distribution and realignment on the nonlinear and inhomogeneous mechanical properties of human supraspinatus tendon under longitudinal tensile loading. *J Orthop Res*. 2009; 27:1596–602. [PubMed: 19544524]
19. Lake SP, Miller KS, Elliott DM, et al. Tensile properties and fiber alignment of human supraspinatus tendon in the transverse direction demonstrate inhomogeneity, nonlinearity and regional isotropy. *J Biomech*. 2010; 43:727–32. [PubMed: 19900677]
20. Clark JM, Harryman DT 2nd. Tendons, ligaments, and capsule of the rotator cuff. Gross and microscopic anatomy. *J Bone Joint Surg Am*. 1992; 74:713–25. [PubMed: 1624486]
21. Reilly P, Amis AA, Wallace AL, et al. Mechanical factors in the initiation and propagation of tears of the rotator cuff. Quantification of strains of the supraspinatus tendon in vitro. *J Bone Joint Surg Br*. 2003; 85:594–9. [PubMed: 12793570]
22. Badylak SF, Gilbert TW. Immune Response to Biologic Scaffold Materials. *Semin Immunol*. 2008; 20:109–16. [PubMed: 18083531]
23. Bolgen N, Menciloglu YZ, Acatay K, et al. In vitro and in vivo degradation of non-woven materials made of poly(epsilon-caprolactone) nanofibers prepared by electrospinning under different conditions. *J Biomater Sci Polym Ed*. 2005; 16:1537–55. [PubMed: 16366336]
24. Moffat KL, Kwei AS, Spalazzi JP, et al. Novel nanofiber-based scaffold for rotator cuff repair and augmentation. *Tissue Eng Part A*. 2009; 15:115–26. [PubMed: 18788982]
25. Lipner J, Shen H, Cavinatto L, et al. In vivo evaluation of adipose derived stromal cells delivered with a nanofiber scaffold for tendon-to-bone repair. *Tissue Eng Part A*. 2015
26. Liu H, Slamovich EB, Webster TJ. Less harmful acidic degradation of poly(lactico-glycolic acid) bone tissue engineering scaffolds through titania nanoparticle addition. *Int J Nanomedicine*. 2006; 1:541–5. [PubMed: 17722285]

27. Moffat KL, Wang IN, Rodeo SA, et al. Orthopedic interface tissue engineering for the biological fixation of soft tissue grafts. *Clin Sports Med.* 2009; 28:157–76. [PubMed: 19064172]
28. Barnes CP, Sell SA, Boland ED, et al. Nanofiber technology: designing the next generation of tissue engineering scaffolds. *Adv Drug Deliv Rev.* 2007; 59:1413–33. [PubMed: 17916396]
29. Leong NL, Kabir N, Arshi A, et al. Use of ultra-high molecular weight polycaprolactone scaffolds for ACL reconstruction. *J Orthop Res.* Oct 24.2015 DOI: 10.1002/jor.23082.
30. Woodruff MA, Hutmacher DW. The return of a forgotten polymer—Polycaprolactone in the 21st century. *Progress in Polymer Science.* 2010; 35:1217–1256.
31. Dempsey DK, Robinson JL, Iyer AV, et al. Characterization of a resorbable poly(ester urethane) with biodegradable hard segments. *J Biomater Sci Polym Ed.* 2014; 25:535–54. [PubMed: 24483140]
32. Tovar N, Bourke S, Jaffe M, et al. A comparison of degradable synthetic polymer fibers for anterior cruciate ligament reconstruction. *J Biomed Mater Res A.* 2010; 93:738–47. [PubMed: 19623532]
33. Macri LK, Sheihel L, Singer AJ, et al. Ultrafast and fast bioerodible electrospun fiber mats for topical delivery of a hydrophilic peptide. *J Control Release.* 2012; 161:813–20. [PubMed: 22580116]
34. Musson DS, Naot D, Chhana A, et al. In vitro evaluation of a novel non-mulberry silk scaffold for use in tendon regeneration. *Tissue Eng Part A.* 2015; 21:1539–51. [PubMed: 25604072]
35. Chainani A, Hippensteel KJ, Kishan A, et al. Multilayered electrospun scaffolds for tendon tissue engineering. *Tissue Eng Part A.* 2013; 19:2594–604. [PubMed: 23808760]
36. Orr SB, Meehan S, Chainani A, Enayet N, Hippensteel KJ, Ruch DS, Guilak F, Little D. Poly(ε)caprolactone-Tendon Derived Matrix Multi-Layered Electrospun Scaffolds for Rotator Cuff Tendon Tissue Engineering. *Proc Orthoped Res Soc.* 2014; 39:507.
37. Zhang X, Bogdanowicz D, Eriskin C, et al. Biomimetic scaffold design for functional and integrative tendon repair. *J Shoulder Elbow Surg.* 2012; 21:266–77. [PubMed: 22244070]
38. Valizadeh A, Mussa Farkhani S. Electrospinning and electrospun nanofibres. *IET Nanobiotechnol.* 2014; 8:83–92. [PubMed: 25014079]
39. Mauck RL, Baker BM, Nerurkar NL, et al. Engineering on the Straight and Narrow: The Mechanics of Nanofibrous Assemblies for Fiber-Reinforced Tissue Regeneration. *Tissue Eng Part B Rev.* 2009; 15:171–93. [PubMed: 19207040]
40. Ayres C, Bowlin GL, Henderson SC, et al. Modulation of anisotropy in electrospun tissue-engineering scaffolds: Analysis of fiber alignment by the fast Fourier transform. *Biomaterials.* 2006; 27:5524–34. [PubMed: 16859744]
41. Baker SC, Atkin N, Gunning PA, et al. Characterisation of electrospun polystyrene scaffolds for three-dimensional in vitro biological studies. *Biomaterials.* 2006; 27:3136–46. [PubMed: 16473404]
42. Garrigues NW, Little D, O'Connor CJ, et al. Use of an insulating mask for controlling anisotropy in multilayer electrospun scaffolds for tissue engineering. *J Mater Chem.* 2010; 20:8962–8968. [PubMed: 21072247]
43. Li D, Ouyang G, McCann JT, et al. Collecting Electrospun Nanofibers with Patterned Electrodes. *Nano Letters.* 2005; 5:913–916. [PubMed: 15884893]
44. Li WJ, Mauck RL, Cooper JA, et al. Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering. *J Biomech.* 2007; 40:1686–93. [PubMed: 17056048]
45. Lim JM, Moon JH, Yi GR, et al. Fabrication of one-dimensional colloidal assemblies from electrospun nanofibers. *Langmuir.* 2006; 22:3445–9. [PubMed: 16584206]
46. Liu Y, Zhang X, Xia Y, et al. Magnetic-Field-Assisted Electrospinning of Aligned Straight and Wavy Polymeric Nanofibers. *Advanced Materials.* 2010; 22:2454–2457. [PubMed: 20376855]
47. Orr SB, Chainani A, Hippensteel KJ, et al. Aligned multilayered electrospun scaffolds for rotator cuff tendon tissue engineering. *Acta Biomater.* 2015; 25:117–26. [PubMed: 26079676]
48. Salalha W, Dror Y, Khalfin RL, et al. Single-walled carbon nanotubes embedded in oriented polymeric nanofibers by electrospinning. *Langmuir.* 2004; 20:9852–5. [PubMed: 15491224]

49. Sell SA, McClure MJ, Ayres CE, et al. Preliminary Investigation of Airgap Electrospun Silk-Fibroin-Based Structures for Ligament Analogue Engineering. *Journal of Biomaterials Science, Polymer Edition*. 2011; 22:1253–1273. [PubMed: 20615358]
50. Theron A, Zussman E, Yarin AL. Electrostatic field-assisted alignment of electrospun nanofibres. *Nanotechnology*. 2001; 12:384.
51. Yang D, Lu B, Zhao Y, et al. Fabrication of Aligned Fibrous Arrays by Magnetic Electrospinning. *Advanced Materials*. 2007; 19:3702–3706.
52. Yang D, Zhang J, Zhang J, et al. Aligned electrospun nanofibers induced by magnetic field. *Journal of Applied Polymer Science*. 2008; 110:3368–3372.
53. Yang F, Murugan R, Wang S, et al. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials*. 2005; 26:2603–10. [PubMed: 15585263]
54. Zhou W, Li Z, Zhang Q, et al. Gas flow-assisted alignment of super long electrospun nanofibers. *J Nanosci Nanotechnol*. 2007; 7:2667–73. [PubMed: 17685282]
55. Khorshidi S, Solouk A, Mirzadeh H, et al. A review of key challenges of electrospun scaffolds for tissue-engineering applications. *J Tissue Eng Regen Med*. Jan 26.2015 doi: 10.1002/term.1978.
56. Khil MS, Bhattarai SR, Kim HY, et al. Novel fabricated matrix via electrospinning for tissue engineering. *J Biomed Mater Res B Appl Biomater*. 2005; 72:117–24. [PubMed: 15389495]
57. Kidoaki S, Kwon IK, Matsuda T. Mesoscopic spatial designs of nano- and microfiber meshes for tissue-engineering matrix and scaffold based on newly devised multilayering and mixing electrospinning techniques. *Biomaterials*. 2005; 26:37–46. [PubMed: 15193879]
58. Pham QP, Sharma U, Mikos AG. Electrospun poly(epsilon-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules*. 2006; 7:2796–805. [PubMed: 17025355]
59. Shang S, Yang F, Cheng X, et al. The effect of electrospun fibre alignment on the behaviour of rat periodontal ligament cells. *Eur Cell Mater*. 2010; 19:180–92. [PubMed: 20419630]
60. Smit E, B ttner U, Sanderson RD. Continuous yarns from electrospun fibers. *Polymer*. 2005; 46:2419–2423.
61. Stachewicz U, Bailey RJ, Zhang H, et al. Wetting Hierarchy in Oleophobic 3D Electrospun Nanofiber Networks. *ACS Appl Mater Interfaces*. 2015; 7:16645–52. [PubMed: 26176304]
62. Truong YB, Glattauer V, Briggs KL, et al. Collagen-based layer-by-layer coating on electrospun polymer scaffolds. *Biomaterials*. 2012; 33:9198–204. [PubMed: 23036963]
63. Tzezana R, Zussman E, Levenberg S. A layered ultra-porous scaffold for tissue engineering, created via a hydrospinning method. *Tissue Eng Part C Methods*. 2008; 14:281–8. [PubMed: 18781888]
64. Yang X, Shah JD, Wang H. Nanofiber enabled layer-by-layer approach toward three-dimensional tissue formation. *Tissue Eng Part A*. 2009; 15:945–56. [PubMed: 18788981]
65. Buckley MR, Evans EB, Matuszewski PE, et al. Distributions of types I, II and III collagen by region in the human supraspinatus tendon. *Connect Tissue Res*. 2013; 54:374–9. [PubMed: 24088220]
66. Tseng T MS, Chainani A, Orr SB, Gilchrist CL, Little D. Biaxially Aligned Tendon-derived Matrix-Poly(E-caprolactone) (PCL) Electrospun Scaffolds For Rotator Cuff Tendon Tissue Engineering. *Proc Orthoped Res Soc*. 2015; 40:2002.
67. Smith L, Xia Y, Galatz LM, et al. Tissue-engineering strategies for the tendon/ligament-to-bone insertion. *Connect Tissue Res*. 2012; 53:95–105. [PubMed: 22185608]
68. Huang AH, Lu HH, Schweitzer R. Molecular regulation of tendon cell fate during development. *Journal of Orthopaedic Research*. 2015; 33:800–812. [PubMed: 25664867]
69. Dymnt NA, Breidenbach AP, Schwartz AG, et al. Gdf5 progenitors give rise to fibrocartilage cells that mineralize via hedgehog signaling to form the zonal enthesis. *Dev Biol*. 2015; 405:96–107. [PubMed: 26141957]
70. Font Tellado S, Balmayor ER, Van Griensven M. Strategies to engineer tendon/ligament-to-bone interface: Biomaterials, cells and growth factors. *Adv Drug Deliv Rev*. 2015

71. Peterson DR, Ohashi KL, Aberman HM, et al. Evaluation of a collagen-coated, resorbable fiber scaffold loaded with a peptide basic fibroblast growth factor mimetic in a sheep model of rotator cuff repair. *J Shoulder Elbow Surg.* 2015; 24:1764–73. [PubMed: 26251198]
72. Tokunaga T, Shukunami C, Okamoto N, et al. FGF-2 Stimulates the Growth of Tenogenic Progenitor Cells to Facilitate the Generation of Tenomodulin-Positive Tenocytes in a Rat Rotator Cuff Healing Model. *Am J Sports Med.* 2015; 43:2411–22. [PubMed: 26311443]
73. Tokunaga T, Ide J, Arimura H, et al. Local Application of Gelatin Hydrogel Sheets Impregnated With Platelet-Derived Growth Factor BB Promotes Tendon-to-Bone Healing After Rotator Cuff Repair in Rats. *Arthroscopy.* 2015; 31:1482–91. [PubMed: 25911389]
74. Dines JS, Grande DA, Dines DM. Tissue engineering and rotator cuff tendon healing. *J Shoulder Elbow Surg.* 2007; 16:S204–7. [PubMed: 17524676]
75. Montgomery SR, Petrigliano FA, Gamradt SC. Biologic augmentation of rotator cuff repair. *Curr Rev Musculoskelet Med.* 2011; 4:221–30. [PubMed: 22021012]
76. Tao X, Liu J, Chen L, et al. EGR1 induces tenogenic differentiation of tendon stem cells and promotes rabbit rotator cuff repair. *Cell Physiol Biochem.* 2015; 35:699–709. [PubMed: 25592085]
77. Sun L, Li H, Qu L, et al. Immobilized Lentivirus Vector on Chondroitin Sulfate-Hyaluronate Acid-Silk Fibroin Hybrid Scaffold for Tissue-Engineered Ligament-Bone Junction. *BioMed Research International.* 2014; 2014:10.
78. Min HK, Oh SH, Lee JM, et al. Porous membrane with reverse gradients of PDGF-BB and BMP-2 for tendon-to-bone repair: in vitro evaluation on adipose-derived stem cell differentiation. *Acta Biomater.* 2014; 10:1272–9. [PubMed: 24370639]
79. Rowland CR, Little D, Guilak F. Factors influencing the long-term behavior of extracellular matrix-derived scaffolds for musculoskeletal soft tissue repair. *J Long Term Eff Med Implants.* 2012; 22:181–93. [PubMed: 23582110]
80. Garrigues NW, Little D, Sanchez-Adams J, et al. Electrospun cartilage-derived matrix scaffolds for cartilage tissue engineering. *J Biomed Mater Res A.* 2014; 102:3998–4008. [PubMed: 24375991]
81. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Semin Immunol.* 2008; 20:86–100. [PubMed: 18162407]
82. Smith MJ, Smith DC, Bowlin GL, et al. Modulation of murine innate and acquired immune responses following in vitro exposure to electrospun blends of collagen and polydioxanone. *J Biomed Mater Res A.* 2010; 93:793–806. [PubMed: 19653305]
83. Cao H, McHugh K, Chew SY, et al. The topographical effect of electrospun nanofibrous scaffolds on the in vivo and in vitro foreign body reaction. *J Biomed Mater Res A.* 2010; 93:1151–9. [PubMed: 19768795]
84. Leigh DR, Mesiha M, Baker AR, et al. Host response to xenograft ECM implantation is not different between the shoulder and body wall sites in the rat model. *J Orthop Res.* 2012; 30:1725–31. [PubMed: 22618690]
85. Thomopoulos S, Parks WC, Rifkin DB, et al. Mechanisms of tendon injury and repair. *J Orthop Res.* 2015; 33:832–9. [PubMed: 25641114]
86. Derwin KA, Baker AR, Iannotti JP, et al. Preclinical models for translating regenerative medicine therapies for rotator cuff repair. *Tissue Eng Part B Rev.* 2010; 16:21–30. [PubMed: 19663651]
87. Ji X, Bao N, An KN, et al. A Canine Non-Weight-Bearing Model with Radial Neurectomy for Rotator Cuff Repair. *PLoS One.* 2015; 10:e0130576. [PubMed: 26107616]
88. Islam A, Bohl MS, Tsai AG, et al. Biomechanical evaluation of a novel suturing scheme for grafting load-bearing collagen scaffolds for rotator cuff repair. *Clin Biomech (Bristol, Avon).* 2015; 30:669–75.
89. Beason DP, Connizzo BK, Dourte LM, et al. Fiber-aligned polymer scaffolds for rotator cuff repair in a rat model. *J Shoulder Elbow Surg.* 2012; 21:245–50. [PubMed: 22244068]
90. Pedowitz RA, Yamaguchi K, Ahmad CS, et al. American Academy of Orthopaedic Surgeons Clinical Practice Guideline on: optimizing the management of rotator cuff problems. *J Bone Joint Surg Am.* 2012; 94:163–7. [PubMed: 22258004]

Figure 1.

(A) Unseeded multi-layered nanofibrous scaffolds electrospun from tendon-derived matrix (porcine tendon) demonstrate retention of collagen staining as assessed by Fast Green. By 14 days after seeding with human adipose stem cells, cells ⁹⁰ (B) have infiltrated through the full thickness of the scaffold, and early evidence of new collagen matrix synthesis through the full thickness of the scaffold (C) is identified by Fast Green staining. Bar = 100μm.