J Shoulder Elbow Surg (2012) 21, 266-277

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

Biomimetic scaffold design for functional and integrative tendon repair

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Rotator cuff tears represent the most common shoulder injuries in the United States. The debilitating effect of this degenerative condition coupled with the high incidence of failure associated with existing graft choices underscores the clinical need for alternative grafting solutions. The 2 critical design criteria for the ideal tendon graft would require the graft to not only exhibit physiologically relevant mechanical properties but also be able to facilitate functional graft integration by promoting the regeneration of the native tendon-to-bone interface. Centered on these design goals, this review will highlight current approaches to functional and integrative tendon repair. In particular, the application of biomimetic design principles through the use of nanofiber- and nanocomposite-based scaffolds for tendon tissue engineering will be discussed. This review will begin with nanofiber-based approaches to functional tendon repair, followed by a section highlighting the exciting research on tendon-to-bone interface regeneration, with an emphasis on implementation of strategic biomimicry in nanofiber scaffold design and the concomitant formation of graded multi-tissue systems for integrative soft-tissue repair. This review will conclude with a summary and discussion of future directions.

Level of evidence: Review Article.

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Keywords: Tendon; bone; interface; insertion; nanofiber; hydroxyapatite; biomimetic; tissue engineering

Rotator cuff tears are among the most common injuries afflicting the shoulder, and in the United States alone, over 250,000 cuff repairs are performed annually.[34] Clinical intervention is required because these tendon injuries do not heal, largely because of the complex anatomy and extended range of motion of the shoulder joint, as well as hypovascularization of the cuff tendons and relative weakening with degeneration.[13,18,34,93] Early primary anatomic repair followed by carefully controlled rehabilitation is currently the standard treatment for rotator cuff tears.[18] Advances in surgical techniques coupled with mechanical fixation methods have significantly improved biomechanical strength and graft stability after repair.[67] As such, failure rates between 20% and 90% have been reported after primary repair of chronic rotator cuff injuries,[26] attributed to factors such as degenerative and poorly vascularized tendons, muscle atrophy, and lack of graft-to-bone integration.[28,30,56,75,76] These problems are exacerbated by the limited healing potential of the injured tissue, relative scarcity of autografts, and potential risks associated with allografts.

Institutional review board: not applicable (review article).

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1058-2746/$ - see front matter © 2012 Journal of Shoulder and Elbow Surgery Board of Trustees.
doi:10.1016/j.jse.2011.11.016

To improve healing, biologic or synthetic polymer-based tendon grafts or augmentation devices[65,70] have been explored to reconstruct large rotator cuff defects but with limited success. To date, extracellular matrix (ECM)–derived scaffolds have been the most commonly used grafts to augment rotator cuff repair.[19] Graft patches or tendon onlays based on decellularized allogeneic and xenogeneic ECM[2,14,18,20,80] provide both mechanical augmentation and the biologic cues to improve healing while also maintaining the ability to be remodeled by host cells. Small intestinal submucosa (SIS), containing a collagen nanofiber–based architecture and alignment, is commercially available as a graft patch for improving cuff repair. Although promising results have been reported in animal models, suboptimal outcomes were observed in human trials,[37,81] attributed to a mismatch in mechanical properties and rapid matrix remodeling experienced in the physiologically demanding and often diseased shoulder joint. A systematic comparison of 4 commercially available ECM scaffolds—Restore, made from porcine SIS; CuffPatch, made from porcine SIS; GraftJacket, made from human dermis; and TissueMend, made from bovine dermis—was conducted by Derwin et al[20] using a canine model. They found that SIS scaffolds had significantly lower mechanical properties than the native tendon; also noted was a decrease in mechanical properties due to premature graft resorption. Therefore, the debilitating effect of rotator cuff tears coupled with the high incidence of failure associated with existing graft choices underscores the clinical need for alternative grafting solutions with physiologically relevant mechanical properties.

In addition to the aforementioned functional requirements for tendon grafting, another challenge in tendon repair arises from the need for biologic fixation of the tendon graft. It has been observed that full-thickness rotator cuff tears most often result from avulsion of the supraspinatus tendon from the humeral head at the insertion site,[13] thereby requiring tendon-to-bone repair. The supraspinatus tendon inserts into the humeral head via a direct enthesis exhibiting region-dependent matrix heterogeneity and mineral content. Specifically, 4 distinct yet continuous tissue regions are observed at the tendon-bone interface (Fig. 1): tendon proper, nonmineralized fibrocartilage, mineralized fibrocartilage, and bone.[6,7,91] The tendon proper consists of fibroblasts found between aligned collagen fibers in a matrix rich in type I collagen, with small amounts of type III collagen and proteoglycans.[10] The nonmineralized fibrocartilage region is composed of fibrochondrocytes in a matrix of type I, type II, and type III collagen with fibers oriented perpendicular to the calcified interface region.[43] The mineralized fibrocartilage region consists of hypertrophic fibrochondrocytes within a matrix of type I and type II collagen,[43] as well as type X collagen.[87] The last region of the insertion site is bone, which consists of osteoblasts, osteoclasts, and osteocytes in a mineralized matrix rich in type I collagen. This multi-tissue organization mediates load transfer between tendon and bone,[6,91] minimizes the formation of stress concentrations,[6,60,90] and supports the heterotypic cellular interactions necessary for interface function and homeostasis.[54] Published studies evaluating tendon-to-bone healing have shown that the normal insertion site is not regenerated after cuff repair based on current mechanical fixation methods,[31,74] and this lack of tendon-bone integration remains a primary cause of repair failure. More recently, given the aforementioned limitations associated with available grafting options, by combining cells, growth factors, and/or biomaterials, the principles of tissue engineering[46,58] have been applied to the formation of tendon-like[29,33,39,59] or bone-like[58,95] tissues in vitro and in vivo, with promising results. As such, the critical barrier to their clinical application is in how to achieve biologic fixation of these newly formed grafts either with each other or with the host environment (or both).[54,61]

These observations collectively suggest that, for functional and integrative repair of rotator cuff injuries, the 2 critical design criteria for the ideal tendon graft would center on controlling scaffold design such that the graft will exhibit physiologically relevant mechanical properties in addition to facilitating the regeneration of the tendon-to-bone interface. With a focus on these design considerations, the objective of this review is to provide an overview of current approaches to functional and integrative tendon repair. In particular, the application of biomimetic scaffold design or, more specifically, the use of nanofiber- and nanocomposite-based scaffolds for tendon tissue engineering will be reviewed. Scaffolds provide a framework for cells to attach, proliferate, and produce matrix and can also serve as carriers for cells and the biomolecules necessary to guide and accelerate healing. To date, nanofibers have been widely investigated for the regeneration of a variety of connective tissues, such as bone,[27,97] meniscus,[3] intervertebral disk,[64] cartilage,[49] and ligament.[5,48] In addition to being biomimetic with respect to the collagenous matrix (Fig. 1), a distinct advantage of nanofiber scaffolds is that they can be engineered to resemble the native tendon ECM, exhibiting high aspect ratio, surface area, permeability, and porosity.[12,50,55,63,69] Moreover, nanofiber organization and alignment can be modulated during fabrication,[63,68] which allows for scaffold structural and material properties to be readily tailored to meet the functional demands of the rotator cuff tendons. Matrix anisotropy can be incorporated into scaffold design with high fidelity by controlling nanofiber organization and alignment. This is especially desirable for functional and integrative tendon repair, because scaffolds with biomimetic anisotropy can be fabricated to recapitulate the inherent structure-function relationship of the rotator cuff tendons, as well as at the tendon-to-bone interface.

This article will begin with a review of nanofiber-based approaches for functional tendon repair (Table I), followed by a section highlighting the exciting research on tendon-to-bone interface regeneration and biologic fixation

Tissue Region	Cell Type	Major Matrix Component
Tendon	Fibroblasts	Collagen types I, III (Diameter: 40-400 nm)*
Non-mineralized Fibrocartilage	Fibrochondrocytes	Collagen types I, II, III
Mineralized Fibrocartilage	Hypertrophic Fibrochondrocytes	Collagen types I, II, X
Bone	Osteoblasts Osteocytes Osteoclasts	Collagen type I (Diameter: 34.5-39.5nm)^

Figure 1 Structure and composition of tendon-to-bone insertion site with Masson trichrome staining in a rat.[62] *Asterisk*, Liang et al[52] (2006); *caret*, Tzaphlidou (2008).

(Table II), with an emphasis on implementation of strategic biomimicry in nanofiber scaffold design and the concomitant formation of graded multi-tissue systems for integrative soft-tissue repair. Finally, a summary will conclude the review, and future directions will be discussed.

Current approaches to functional tendon repair

Nanofiber scaffold design and modification for tendon tissue engineering

The ideal scaffold for functional and integrative tendon repair must first meet the physiologic demands of the native tendon by matching its mechanical properties while simultaneously promoting host cell–mediated healing by mimicking the ultrastructural organization of the native tendon. Furthermore, the scaffold should be biodegradable to be gradually replaced by new tissue while maintaining its physiologically relevant mechanical properties. Lastly, the scaffold must integrate with the host tendon and surrounding bone tissue by promoting the regeneration of the native tendon-to-bone interface. It is well established that the highly organized nanoscale structure of tendons is characterized by closely packed parallel collagen fiber bundles, varying in diameter, and is composed of bundles of individual collagen fibrils approximately 1 to 2 nm in diameter (Fig. 1). This structural arrangement is critical for the physiologic function of tendons, which includes the stabilization and guidance of joint motion, transmission of physiologic loads, and maintenance of the anatomic alignment of the skeleton. Furthermore, the parallel alignment of collagen fibers along the direction of applied load results in one of the strongest tissues in the body.[42] The collagen fibers of tendons and ligaments typically exhibit a bimodal diameter distribution in the nanometer range (approximately 40-400 nm) that varies according to the specific tissue type, as well as among individuals, and may also be altered during scar formation after injury.[52]

Several groups have explored tissue engineering methods for tendon or ligament repair.[1,15,21,53] Synthetic as well as biologically derived grafts have shown favorable results during in vitro culture trials, as well as in relevant in vivo models. It is common to use scaffolds composed of microfibers based on a variety of synthetic polymers, such as poly-L-lactic acid (PLLA), polylactide-co-glycolide (PLGA), and polyurethane,[15,53] as well as biologic materials, such as collagen[21] and silk.[1,36] Although these approaches have shown promising results, the scaffold architecture differs significantly from that of the inherent nanoscale organization of tendons or ligaments. Given that scaffold fiber diameters have been shown to directly affect fibroblast phenotype and matrix production,[5] there is significant interest in enhancing physiologically relevant soft-tissue regeneration using scaffolds that more closely mimic the native tissue nanostructure and mechanics.

The nanoscale architecture of the collagen-rich tendon matrix can be readily recapitulated with nanofiber scaffolds, which exhibit a high surface area–to–volume ratio, low density, high porosity, variable pore size, and mechanical properties approximating those of the native tissues. Nanofibers can be fabricated with a variety of methods,[44] such as drawing, template synthesis, temperature-induced phase separation, molecular self-assembly, and most frequently, electrospinning.[57,72] Moffat et al[59] were the first authors to report on the fabrication of PLGA nanofiber scaffolds with physiologically relevant structural and mechanical properties for rotator cuff repair. They observed that human rotator cuff fibroblast morphology and growth on aligned (mean fiber diameter, 615 nm) and unaligned (mean fiber diameter, 568 nm) fiber matrices were dictated by fiber alignment, with distinct cell morphology and integrin expression profiles. Upregulation of α2 integrin, a key mediator of cellular attachment to collagenous matrices, was observed when the fibroblasts were cultured on aligned fibers and upon which a type I and type III collagen–rich matrix was deposited. More recently,

Table I Scaffold-based approaches for tendon repair

Study	Scaffold composition	Scaffold design	Cell type/evaluation	Observations
Gilbert et al[32] (2007)	Porcine SIS	Homogeneous	NIH 3T3 fibroblasts In vitro	Type I collagen increases and type III collagen decreases with increased frequency of stretch
Juncosa-Melvin et al[41] (2006)	Collagen sponge (type I)	Homogeneous	hMSCs In vitro and in vivo (rat patellar tendon defect model)	Mechanical stimulation improves linear stiffness
Sahoo et al[78] (2006)	PLGA nanofibers (300-900 nm) vs PLGA microfibers	Variable fiber diameter	Porcine MSCs In vitro	Cell attachment comparable to fibrin gel–microfiber control; gene expression indicates capacity to differentiate toward tendon lineage
Barber et al[4] (2011)	Braided aligned PLGA nanofiber (702 $\pm$ 205 nm)	3, 4, or 5 aligned nanofiber bundles	hMSCs In vitro	Abundant matrix formation and upregulation of scleraxis, indicating differentiation into tenogenic lineage
Sahoo et al[77] (2010)	PLGA nanofiber (200-700 nm) with FGF incorporated + silk microfiber	Unaligned PLGA nanofibers with electrospun onto knitted silk fibers	Rabbit BMSCs In vitro	Scaffolds stimulate MSC proliferation; gene expression indicates tenogenic differentiation
Yin et al[96] (2010)	PLLA aligned nanofiber (430 $\pm$ 170 nm) vs unaligned nanofiber (450 $\pm$ 110 nm)	Homogeneous	Human TSPCs In vitro and in vivo (intramuscular implantation in mouse model)	Aligned scaffolds promote expression of tenogenic markers; in vivo, aligned scaffolds guide cell and matrix organization
Pham et al[69] (2006)	Micro (2-10 μm) or nano (615 $\pm$ 152 nm)-microfibrous PCL	Variable fiber diameter	Rat MSCs In vitro	Culture in flow perfusion bioreactor enhances MSC infiltration distance through scaffold
Srouji et al[83] (2008)	Unaligned PCL and collagen (1:1)	Homogeneous	hMSCs In vitro and in vivo (subcutaneous, nude mouse model)	Plug-flow bioreactor culture enhances cell proliferation and infiltration; integration with host tissue and neovascularization in vivo
Moffat et al[59] (2009)	PLGA aligned nanofiber (615 $\pm$ 152 nm) vs unaligned nanofiber (568 $\pm$ 147 nm)	Homogeneous	Human rotator cuff tendon fibroblasts In vitro	Cell morphology and matrix alignment governed by fiber alignment

BMSC, bone mesenchymal stem cell; *FGF*, fibroblast growth factor; *MSC*, mesenchymal stem cell.

Table II Scaffold-based approaches for tendon-to-bone interface regeneration

Study	Scaffold composition	Scaffold design	Cell type/evaluation	Observations
Erisken et al[22] (2008)	Unaligned, extruded/electrospun PCL nanofibers (200–2,000 nm)	Gradient of scaffold mineral content	Mouse preosteoblastic cells (MC3T3) In vitro	Formation of gradient of calcified matrix
Li et al[51] (2009)	Gelatin-coated PCL nanofibers and plasma-treated PLGA	Graded coating of calcium phosphate	Mouse preosteoblastic cells (MC3T3) In vitro	Cells preferentially adhere and proliferate on areas with high CP content
Xie et al[92] (2010)	Aligned and unaligned PLGA nanofibers	Aligned-to-random fiber orientation	Rat tendon fibroblasts In vitro	Cells align/organize on aligned fibers; cells remain unorganized on random fibers
Moffat et al[62] (2011)	Aligned PLGA deposited over PLGA-hydroxyapatite nanofibers Phase A (615 ± 152 nm) Phase B (340 ± 77 nm)	Biphasic with contiguous layer of PLGA and PLGA-hydroxyapatite	Bovine chondrocytes In vitro Rat BMSCs In vivo rat rotator cuff model	Contiguous noncalcified and calcified fibrocartilage-like matrix was formed in vitro and in vivo

BMSC, bone mesenchymal stem cell.

Xie et al[92] developed a single continuous PLGA nanofiber scaffold that transitioned from aligned to random orientation to examine the effects of this transitional region on rat tendon fibroblasts in vitro. After 7 days of culture, the study showed that cells proliferated on both aligned and random nanofiber orientations but that a rounded morphology was found on unaligned nanofibers; though similar to what was seen by Moffat et al, cells cultured on aligned nanofibers appeared long and spindle-like and were aligned along the long axes of the fibers.

Biologic response to polymeric nanofibers may also be enhanced by additional surface modifications. For example, Rho et al[73] electrospun aligned type I collagen nanofiber scaffolds with a mean fiber diameter of 460 nm and evaluated the response of human epidermal cells after coating the scaffolds with several adhesion proteins. They found that cell proliferation was enhanced by coating the scaffolds with both type I collagen and laminin. Recently, Park et al[66] applied plasma treatment to polyglycolic acid, PLGA, and PLLA nanofibers and grafted a surface layer of hydrophilic acrylic on these scaffolds. They found that NIH 3T3 fibroblasts seeded on these modified scaffolds spread and proliferated faster compared with those on unmodified controls. Another biomimetic method to enhance biologic responses is to incorporate collagen into nanofiber scaffolds. Using co-electrospinning, Zhang et al[98] added collagen to poly-ε-caprolactone (PCL) nanofibers, in addition to coating these nanofibers with collagen. Although these scaffolds promoted human dermal fibroblast growth independent of the incorporation method, cell migration into the scaffold was mainly observed in the co-electrospun PCL-collagen scaffolds. Similarly, Theisen et al[85] seeded human tendon fibroblasts on a composite PLLA–type I collagen scaffold and found that, when compared with the PLLA control, the blended scaffold upregulated the expression of type I, type III, and type X collagen and decorin.

Nanofibers have also been used to improve existing scaffold design, resulting in a graft with a more biomimetic surface for eliciting desired cell response. For example, Sahoo et al[78] electrospun PLGA nanofibers directly onto a woven microfiber PLGA scaffold to increase cell seeding efficiency while maintaining a scaffold that was mechanically competent. The attachment, proliferation, and differentiation of porcine bone marrow stromal cells were evaluated on these scaffolds, and when compared with scaffolds seeded through a fibrin gel delivery, cells seeded onto nanofiber-coated scaffolds enhanced proliferation and collagen production and upregulated the gene expression of several tendon-related markers, namely decorin, biglycan, and type I collagen.

In an alternative strategy to enhance the mechanical properties of electrospun nanofibers, Barber et al[4] fabricated braided nanofiber scaffolds by electrospinning bundles of aligned PLLA and braiding either 3, 4, or 5 bundles together to generate their final construct. Human mesenchymal stem cells (hMSCs) cultured on the braided scaffolds aligned parallel to the length of the nanofibers and displayed realignment of actin filaments, which progressed with culture time. Cells produced a matrix that bridged the gap between bundles, and when the hMSCs were concurrently stimulated with cyclic tensile strain and cultured in tenogenic medium containing bone morphogenetic protein 2, differentiation factor 5, and fibroblast growth factor 2, a significant upregulation of scleraxis was reported, indicative of hMSC differentiation into the tenogenic lineage.

The incorporation of bioactive molecules in the scaffold system to promote stem cell differentiation is another strategy adopted for tendon repair. Recently, Sahoo et al[77] seeded rabbit mesenchymal progenitor cells on a hybrid scaffold for tendon repair. The scaffold was fabricated by electrospinning basic fibroblast growth factor–releasing PLGA nanofibers onto knitted silk microfibers. This novel scaffold mimicked the ECM in function, initially stimulating stem cell proliferation and subsequently promoting tenogenic differentiation as indicated by an increase in both type I and type III collagen expression after 2 weeks in vitro.

The recent identification of tendon stem cells (TSPCs)[9] has provided another cell source for studying tendon development and repair. Human TSPCs typically reside in a matrix of parallel collagen fibers; thus, fiber alignment is expected to play a role in regulating stem cell differentiation.[9,35] When Yin et al[96] investigated the impact of PLLA nanofiber alignment on human TSPC differentiation, the expression of the tendon-specific gene scleraxis, as well as the matrix gene collagen XIV, was significantly higher on aligned versus random scaffolds after 7 days of culturing in osteogenic media. On the other hand, both gene expression and histologic staining indicated that the randomly orientated nanofibers stimulate human TSPC differentiation toward an osteogenic lineage, whereas this was not observed for the aligned nanofiber group, providing evidence that cell orientation induced by the scaffold nanotopography plays an important role in cell differentiation. Finally, intramuscular evaluation of human TSPC–seeded nanofibers in an athymic mouse model showed that the aligned scaffold guided both cell organization and collagen bundle formation, whereas a random orientation of both cells and matrix was observed on the random fiber controls.

Mechanical stimulation of scaffolds

Although limited results are available for mechanical loading on nanofiber-based scaffolds, the role of mechanical loading on tendon tissue engineering has been investigated extensively for collagen-based scaffolds. For example, Gilbert et al[32] loaded NIH 3T3 fibroblasts cultured on porcine SIS-ECM as a function of stretch (0%, 5%, 10%, and 15%) and loading frequencies (0.1, 0.3, and 0.5 Hz). They found that, in general, the expression of type I collagen increased whereas that of type III collagen decreased with increasing frequency, matching collagen expression profiles during the late stage of remodeling during native tendon healing. In another study, Berry et al[8] seeded human dermal fibroblasts in collagen gels and preloaded the gels (2-mN or 10-mN static loading) before applying 10% cyclic strain (1 Hz) for 24 hours. Whereas cell proliferation increased with mechanical loading regardless of preloading regimen, elevated collagen synthesis was only seen in the 2-mN group. Using a custom bioreactor, Garvin et al[29] showed that avian flexor tendon cells seeded on the bioartificial tendon, when subjected to mechanical loading, upregulated type I, type III, and type XII collagen expression at levels consistent with those of cells found in the flexor tendon.

Juncosa-Melvin et al[41] investigated the potential of hMSCs seeded on collagen sponges for patellar tendon repair. Mechanical loading was applied to the scaffold to a peak strain of 4% once every 5 minutes for up to 8 hours per day over a 2-week culture period. The stimulated constructs exhibited 2.5 times the linear stiffness of the non-stimulated controls. When tested in a rabbit patellar tendon defect model accompanied by normal cage activity after surgery, maximum force, linear stiffness, maximum stress, and linear modulus for the stimulated scaffold group were found to approximate those of the native patellar tendon. In a follow-up study, it was found that both type I and type III collagen expression increased significantly in the stimulated group whereas no difference in decorin and fibronectin expression was evident with respect to the unloaded control.[40]

Mechanical stimulation of nanofiber scaffolds with bioreactors has been used to promote cell infiltration through nanofiber scaffolds. Pham et al[68] used a flow perfusion bioreactor and showed that rat mesenchymal stem cell infiltration distance on bilayered constructs of unaligned PCL microfibers with PCL nanofibers on top was increased by a factor of 5 versus the static control. Similarly, Srouji et al[83] used a plug-flow bioreactor to culture hMSCs seeded on unaligned PCL and collagen nanofiber scaffolds. After 6 weeks, cells were evident throughout the scaffold. The preconditioned constructs were implanted subcutaneously in nude mice, and good integration with the surrounding tissues and neovascularization were found. These studies show the ability of media perfusion bioreactor systems to improve mass transport throughout 3-dimensional tissue-engineered constructs and to promote the production of sufficient cell mass necessary for in vivo grafting and host integration.

Soft tissue–to–bone interface regeneration and integrative tendon repair

The debilitating effect of rotator cuff tears coupled with the high incidence of failure associated with existing repair

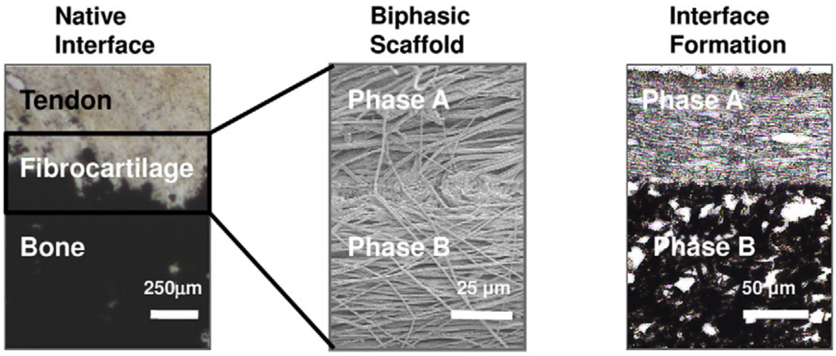

Figure 2 Biomimetic scaffold design. Native tendon-to-bone interface with distinct yet continuous noncalcified and calcified matrix regions (von Kossa staining in a Lewis rat) inspired the design of the biphasic nanofiber scaffold (where phase A is PLGA and phase B is PLGA-hydroxyapatite), which—when tested in vivo—resulted in the formation of noncalcified and calcified matrix regions (subcutaneously at 3 weeks).

techniques[14,20,37] underscores the clinical need for functional solutions for integrative tendon-to-bone repair. The supraspinatus tendon of the rotator cuff connects to bone through a direct insertion, a complex enthesis consisting of 3 distinct yet continuous regions of soft tissue, fibrocartilage, and bone.[6,16,89] The fibrocartilaginous interface region is further divided into noncalcified and calcified regions (Fig. 2). The insertion site serves several functions, including enabling the transfer of loads between distinct tissues,[6,91] minimizing the formation of stress concentrations,[6,60,90] and supporting the communication among multiple cell types necessary for interface function and homeostasis.[54] Therefore, regeneration of this multi-tissue transition is essential for biologic fixation of tendon grafts.

To address this challenge, several groups have evaluated the feasibility of integrating tendon grafts with bone or biomaterials through the formation of anatomic insertion sites. Fujioka et al[24] examined the effects of reattaching the bone and tendon in a rat model of Achilles tendon avulsion. After 4 weeks, surgical reattachment of tendon to bone increased type X collagen deposition and allowed tissue to maintain distinct regions of calcified and noncalcified fibrocartilage tissue. In addition, Inoue et al[38] promoted supraspinatus tendon integration with a metallic implant using a bone marrow–infused bone graft. Other approaches include reattaching the tendon to bone with the aid of natural materials such as periosteum or demineralized bone matrix. Specifically, Chang et al[11] sutured a periosteal flap from tendon to bone at the end of a rabbit infraspinatus tendon and observed remodeling of tissue at the interface over a period of 12 weeks. At 4 weeks after surgery, a fibrous layer with increased mechanical properties was seen at the interface region, which developed into a matrix similar to fibrocartilage after 3 months. This tissue layer possessed an increased failure load, proving improvement in integration at the interface. Similarly, Sundar et al[84] attempted to augment interface healing after surgery by implanting demineralized bone between tendon and bone in an ovine patellar tendon model. They found that bone enhanced deposition of both mineralized and nonmineralized fibrocartilage at the interface, with improved weight bearing. These pioneering studies collectively show the potential for regenerating the tendon-to-bone interface and delineate the need for functional grafting solutions that can promote biologic fixation.

Current knowledge of the structure-function relationship at the tendon-bone insertion[86,87] provides invaluable cues for biomimetic and integrative tendon scaffold design. Combining biomechanical testing with the quasi-linear viscoelastic model,[25] Thomopoulos et al[86,87] determined the mechanical properties of the rat supraspinatus tendon insertion sites and later related them to collagen orientation using a finite element model. They found that controlled collagen fiber alignment plays an important role in reducing stress concentration at the tendon-bone insertion.[86] Another hallmark of the tendon-to-bone interface is a region-dependent mineral distribution across the insertion site.[7,91] Calcium phosphate is a prime modulator of both the biochemical milieu and the nature of mechanical stimuli presented to cells. The presence of the noncalcified and calcified fibrocartilage regions at the interface is of functional significance, because higher matrix mineral content has been associated with greater mechanical properties in connective tissues.[17,23,71] Moffat et al[60] correlated the aforementioned increase in compressive modulus across the interface to the onset of mineral presence in the calcified fibrocartilage region. It is clear that both collagen alignment and mineral content are critical design parameters for functional and integrative tendon repair.

On the basis of these observations, the ideal scaffold for tendon-to-bone interface tissue engineering must exhibit a gradient of structural and mechanical properties mimicking those of the multi-tissue insertion. Compared with a homogeneous structure, a stratified scaffold with predesigned, tissue-specific matrix inhomogeneity can better sustain and transmit the distribution of complex loads inherent at the direct insertion site. A key criterion in stratified scaffold design is that the phases must be interconnected and pre-integrated with each other, thereby supporting the formation of distinct yet continuous multi-tissue regions. In other words, the scaffold would exhibit a gradient of physical properties to allow for the recapitulation of interface-like heterogeneity throughout the scaffold. It should also support growth and differentiation, as well as the interactions between heterotypic and homotypic cell populations, to promote the formation and maintenance of the multi-tissue interface. In addition, the scaffold phases should be biodegradable so that they are gradually replaced by living tissue, and the degradation process must be balanced with respect to mechanical properties to permit physiologic loading and neo-interface function. Finally, the interface scaffold must be compatible with existing tendon reconstruction grafts or pre-incorporated into tissue-engineered graft design to achieve integrative and functional soft-tissue repair.

To this end, a scaffold recapturing the nanoscale interface organization, with preferentially aligned nanofiber organization and region-dependent change in mineral content, would be highly advantageous. Building on the functional PLGA nanofiber scaffold designed for tendon tissue engineering, Moffat et al[59] designed a biphasic scaffold, with the top layer consisting of nanofibers of PLGA and the second layer consisting of composite nanofibers of PLGA and hydroxyapatite nanoparticles. The biphasic design is aimed at regenerating both the nonmineralized and mineralized fibrocartilage regions of the tendon-to-bone insertion site while promoting osteointegration with PLGA-hydroxyapatite nanofibers.[62] The response of tendon fibroblasts, osteoblasts, and chondrocytes was evaluated on these nanocomposite scaffolds with promising results in vitro. When tested in vivo subcutaneously, as well as in a rat rotator cuff repair model,[82] the biphasic scaffold supported regeneration of continuous noncalcified and calcified fibrocartilage regions (Fig. 2), showing the potential of a biodegradable nanofiber–based scaffold system for integrative tendon-to-bone repair.

Controlling scaffold mineral distribution may be another promising approach for repairing the soft tissue–to–bone insertion site. Working with PCL nanofibers and using a novel extrusion system coupled with electrospinning, Erisken et al[22] incorporated calcium phosphate nanoparticles into nonwoven nanofiber meshes, resulting in a gradient of mineral distribution across the depth of the PCL scaffold. Within 4 weeks, culturing of MC3T3 cells on these nanofiber constructs led to the formation of a gradient of calcified matrix. Recently, using the simulated body fluid immersion method, Li et al[51] formed a calcium phosphate coating on a nonwoven mat of gelatin-coated PCL and plasma-treated PLGA nanofibers in a graded manner. They observed that the gradient in mineral content resulted in spatial variations in the stiffness and affected the number of preosteoblastic MC3T3 cells that adhered to the substrate.

In addition to engineering the tendon-bone interface, the muscle-tendon junction is another critical research area for integrative tendon repair. The tendon joins the muscle to bone, and thus, the myotendinous junction, which connects muscle to tendon, acts as a bridge to distribute mechanical loads.[94] This interface consists of a fibroblast-laden, interdigitating band of tissue that connects the dense collagen fibers of the tendon to the more elastic muscle fibers while displaying a gradient of structural properties.[88] Current tissue engineering approaches, as shown by Saxena et al,[79] include the incorporation of myoblasts on a composite scaffold of fibronectin hydrogel and polyglycolic acid. A muscle-like matrix was formed in vitro and was capable of responding to an electrical stimulus. Recently, Larkin et al[47] co-cultured skeletal muscle constructs with engineered tendon constructs to regenerate the muscle-tendon interface. Interestingly, upregulation of paxillin was observed at the neo-interface, and the myotendinous junction formed was able to sustain tensile loading beyond the physiologic strain range. These studies show the promise of the biodegradable nanofiber–based scaffold system for interface tissue engineering and the potential of harnessing cellular interaction for engineering both the tendon-to-bone and muscle-to-tendon interface and, ultimately, functional and integrative tendon repair.[45]

Summary and future directions

Interface tissue engineering focuses on the functional regeneration of the anatomic interface between distinct tissue types to accelerate the translation of tissue-engineered technologies to the clinical setting. It aims to develop innovative technologies for the formation of complex tissue systems, with the broader goal of achieving the biologic fixation of tissue-engineered grafts with the host environment. In this regard, nanotechnology-based approaches to connective tissue repair offer several distinct advantages. Specifically, nanofiber-based scaffold systems are advantageous because of their inherent characteristics, with potential to mimic the native collagenous tendon, interface, and bone matrix and, ultimately, regulate cellular response. In addition, nanofiber substrates can be fabricated from a variety of synthetic as well as natural polymers, with controlled geometry, mechanical properties, porosity, permeability, degradation kinetics, and fiber diameter. The studies highlighted here and many others collectively show the promise and the excitement in the field regarding nanotechnology-based scaffolds for guided orthopedic tissue engineering and integrative soft-tissue repair.

The critical research question in the emerging field of orthopedic interface tissue engineering centers on how the graded structures between different types of connective tissues are formed, re-established after injury, and maintained in the body. Moreover, the effects of biologic, physical, and chemical stimulation on interface formation and regeneration also remain to be explored. It is anticipated that advances in biomimetic design of nanofiber-based scaffolds for integrative soft-tissue repair will be guided by continued exploration of the structure-function relationship of the native tissue-to-tissue interfaces, as well as increased understanding of the mechanisms governing its repair and development.

In addition, scaffold fabrication and scaling issues must be overcome for the widespread clinical utilization of nanofiber-based systems for functional orthopedic tissue engineering and integrative repair to be realized. For example, recent advances in nanotechnology and in the delivery of bioactive agents that are immobilized within the carriers could provide additional methods to control or enhance the formation of single- or multi-tissue systems. However, the electrospinning process used to fabricate nanofibers requires that the polymer first be soluble in a variety of toxic solvents, which may have undesired effects on the incorporation of either biomolecules or cells. Thus, incorporation of biomolecules into nanofiber-based systems, while keeping them structurally stable and biologically active and controlling their subsequent release, remains to be investigated. In addition, high-throughput fabrication and delivery processes need to be developed for scaling up nanofiber scaffolds and enabling their commercial applicability. Moreover, further optimization of scaffold design strategies is anticipated for effective clinical translation and surgical implementation. For example, combining the optimal strategies devised from the tendon and the tendon-to-bone interface regeneration in this review may yield a graft system that can enable integration with both soft and hard tissue. Such a composite scaffold system would be optimal for treating massive rotator cuff tears. Finally, the development of physiologically relevant in vivo soft-tissue repair models, both healthy and diseased, are needed to evaluate the clinical efficacy of these biomimetic scaffolds.

Conclusion

The design of biomimetic, nanofiber-based scaffolds for tendon and tendon-bone interface regeneration described in this review offer a promising strategy for achieving functional and integrative tendon repair. It is anticipated that these efforts will lead to the development of a new generation of biologic fixation devices for soft-tissue repair and will improve clinical outcome, as well as quality of life, for patients experiencing the debilitating effects of soft-tissue injuries.

Disclaimer

The authors gratefully acknowledge the National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR052402, AR056459, and AR055280), New York Stem Cell Initiative (H.H.L.), National Science Foundation Graduate Fellowship (D.B.), GK-12 Fellowship (GK-12 0338329) (N.M.L.), and National Science and Engineering Council of Canada (NSERC PGS-D3) (X.Z.) for funding support.

The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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