HHS Public Access

Author manuscript

Int J Pharm. Author manuscript; available in PMC 2021 June 21.

Published in final edited form as:

Int J Pharm. 2018 June 15; 544(2): 358–371. doi:10.1016/j.ijpharm.2018.01.006.

Growth Factor Delivery Strategies for Rotator Cuff Repair and Regeneration

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Abstract

The high incidence of degenerative tears and prevalence of retears (20–95%) after surgical repair makes rotator cuff injuries a significant health problem. This high retear rate is attributed to the failure of the repaired tissue to regenerate the native tendon-to-bone insertion (enthesis). Biological augmentation of surgical repair such as autografts, allografts, and xenografts are confounded by donor site morbidity, immunogenicity, and disease transmission, respectively. In contrast, these risks may be alleviated via growth factor therapy, which can actively influence the healing environment to promote functional repair. Several challenges have to be overcome before growth factor delivery can translate into clinical practice such as the selection of optimal growth factor(s) or combination, identification of the most efficient stage and duration of delivery, and the design considerations for the delivery device. Emerging insight into the injury-repair microenvironment and our understanding of growth factor mechanisms in healing are informing the design of advanced delivery scaffolds to effectively treat rotator cuff tears. Here, we review potential growth factor candidates, design parameters and material selection for growth factor delivery, innovative and dynamic delivery scaffolds, and novel therapeutic targets from tendon and developmental biology for the structural and functional healing of rotator cuff repair.

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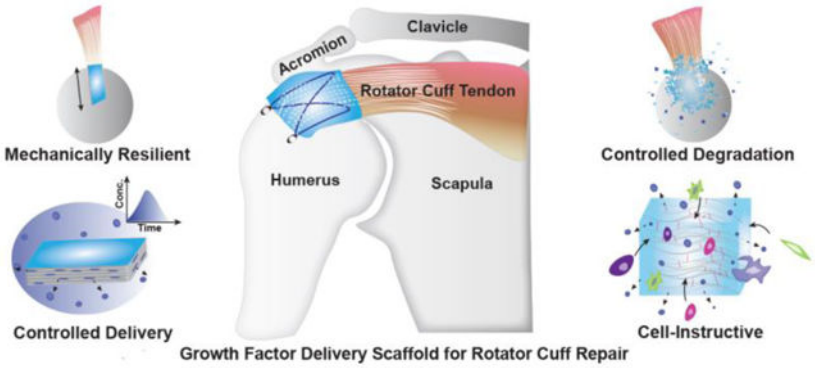
Graphical abstract



Keywords

Rotator cuff; tendon; enthesis; growth factor; delivery; scaffold

1. Introduction

Rotator cuff tears are one of the most leading musculoskeletal injuries in the United States with over 200,000 repair procedures done annually and an estimated $474 million dollars in health care costs (Novakova et al., 2017; Pedowitz et al., 2011). The rotator cuff is a set of muscle-tendon units that stabilize the shoulder joint. It includes the supraspinatus, infraspinatus, subscapularis, and the teres minor and major. These tendons attach to the bone via a specialized tissue called the enthesis, which is a structurally continuous tissue with four transitional zones, fibrous tendon, unmineralized fibrocartilage, mineralized fibrocartilage, and bony attachment (Apostolakos et al., 2014) as illustrated in Figure 1. The chronic degeneration of the enthesis with age is the primary cause of rotator cuff tears but acute tears also occur because of injury.

When a tear occurs, physicians manage the injury both surgically and non-surgically (Harrison and Flatow, 2011) depending on the severity, size, and pattern of the tear. For large and painful tears, or when non-surgical therapy fails to improve painful symptoms in smaller tears, surgical repair is considered. The current method for surgical repair of rotator cuff tears utilizes a single/double row suture technique to re-approximate the torn tendon back to its insertion site on the bone. However, the surgically repaired tendon insertion tissue (enthesis) is prone to high rate of retear between 20–95%, depending upon the extend of tears (Derwin et al., 2010b; Galatz et al., 2004). This high retear rate is attributed to several factors such as age, chronicity of tears, poor vascularization, increased fibrosis, musculo-tendinous retraction, fatty infiltration, peritendinous adhesions, and increased stress concentration at the insertion site (Galatz et al., 2004; Melis et al., 2009; Meyer et al., 2012; Saadat et al., 2016). These factors constitute to the formation of a highly disorganized scar tissue that has poor biomechanical properties. Clinical repair strategies seek to recreate the native enthesis tissue organization (Figure 1.) by reapproximating the torn tendon to its anatomic footprint, providing adequate initial fixation strength for the repair, minimizing

potential gap formation, and maintaining mechanical support until sufficient tissue formation.

Surgical repair of rotator cuff tears is confounded by musculo-tendinous retraction and tendon retears that tend to occur within 12 weeks after surgery (McCarron et al., 2013). To prevent tendon retraction during this early rehabilitative phase, suture protection from intra-tendon movement and sub-acromial bursa friction is desirable. Augmentation of surgical repairs using a patch/scaffold has the potential to protect the suture from this movement and friction, mechanically support the repair, and facilitate biological healing. Patch augmentation is recommended for grade II-V tears, which includes small and medium full thickness tears (< 3 cm involving one tendon) to irreparable large massive tears (3–5 cm involving 2–3 tendons) that are unable to reapproximate to the tuberosity with low tension (Derwin et al., 2010a). Tissue-based and engineered materials have been investigated for patch augmentation.

Tissue-based scaffolds such as autografts, xenografts, and allografts can reinforce the repair by acting as a bridge for cellular infiltration, collagen assembly, and mechanical support. These matrices offer natural porosity, 3-dimensional extracellular matrix cues, and growth signals to promote host tissue integration. Autografts have excellent biocompatibility and graft integration but have limited availability and cause donor-site morbidity. Advances in decellularization techniques have led to improved xenografts (Conexa®) (Lederman et al., 2016) and allografts (Graftjacket®) (Barber et al. 2012), which are more accessible, but they still carry the risk of infection and severe inflammatory reaction from retained source DNA (Walton et al. 2007). These scaffolds also suffer from unpredictable degradation rate, inadequate mechanical properties, and non-specific induction ability (Chen et al., 2009). Such concerns have generated considerable interest in the development of engineered grafts/scaffolds for rotator cuff repair augmentation.

Engineered scaffolds using both natural and synthetic biomaterials and their combinations can provide defined properties for rotator cuff repair. Natural biomaterials such as fibrin, collagen, elastin, and hyaluronic acid present extracellular cues that support cell infiltration and tissue repair, but degrade rapidly and lack the mechanical characteristics necessary for load bearing applications. On the other hand, synthetic non-/biodegradable polymers can be designed to have optimum mechanical properties to support the repaired tissue. Non-biodegradable polymers, although mechanically robust (Ciampi et al. 2014) generally have poor biocompatibility because of frustrated phagocytosis and inflammation. In contrast, biodegradable polymers may provide better biocompatibility. The predictable degradation characteristics of these polymers can be tailored to provide initial mechanical support during the critical rehabilitative phase and slow degradation to permit drug delivery and tissue integration. However, these synthetic scaffolds lack the necessary bioactive cues instructive for tissue repair (Longo et al., 2012). Creation of an instructive environment is significant in the repair of the rotator cuff, which is characterized by a poorly vascularized space, with a majority of degenerative tears seen in the aged population with compromised tissue repair capability. Therefore, tissue repair strategies have sought to incorporate instructive bioactive agents such as cells and/or signaling molecules in biomaterials to augment repair.

Both stem and tissue-typic cell delivery via biomaterials has shown regenerative benefits in rotator cuff healing (Peach et al., 2017) (Funakoshi et al., 2005). Cell delivery faces the challenges of availability, seeding, survival, and specificity, with stem cells facing additional regulatory and ethical barriers. Stem cells and tissue-typic cells are thought to contribute to healing via autocrine/paracrine signaling through growth factors and cytokines. Growth factors delivery from scaffolds; therefore, may be a more simple and direct approach to improve healing outcomes in rotator cuff repair.

Growth factors have the ability to enhance healing response by increasing cell recruitment, proliferation, differentiation, ECM synthesis, and remodeling at the repair site. Several studies have investigated the regenerative potential of growth factors in small animal models and a handful of large animal models (Hee et al., 2011; Novakova et al., 2017; Tokunaga et al., 2015b; Uggen et al., 2010; Zhao et al., 2014). However, growth factor augmented repair, often with better mechanical properties than the control repair groups are still far from achieving the same biomechanical properties as the native enthesis. Several questions need to be addressed to achieve effective growth factor delivery for rotator cuff repair as follows:

- **i.** Which growth factor(s) or combinations of growth factors are needed?
- **ii.** What is the optimum dose and duration of growth factor delivery?
- **iii.** How to deliver these growth factors over a therapeutic time window while maintaining their bioactivity?

Here, we review innovative growth factor delivery strategies to promote rotator cuff repair focusing on promising growth factor candidates, design characteristics and material selection for growth factor delivery scaffolds, and novel therapeutic targets from tendon and developmental biology for rotator cuff repair.

2. Mechanisms of Enthesis Healing and the Role of Growth Factors

Enthesis healing takes place in three phases: inflammatory, reparative, and remodeling (Yang et al., 2013). The inflammatory phase (day 0 to day 7) is characterized by the release of growth factors and pro-inflammatory cytokines needed for the recruitment of inflammatory cells at the site of injury (Reddy et al., 1999). These inflammatory cells phagocytize necrotic tissue and induces further growth factor and cytokine release that is needed for the recruitment and proliferation of cells for repair (Hays et al., 2008). Induction of angiogenic growth factors such as VEGF stimulates blood vessel formation in the repair tissue. In the reparative/formative phase (week 1 to week 8), there is active proliferation of fibroblasts and differentiation of stem cells to relevant tissue-typic cells such as osteoblasts, chondrocytes, and tenocytes (Aicale et al., 2017; Andarawis-Puri et al., 2015; Koike et al., 2005). The proliferated fibroblasts differentially synthesize collagen type I, II, III and X matrix over the following 8-week period. This improves the mechanical strength of the tissue because of the large collagen-based callus formation (Aicale et al., 2017; Andarawis-Puri et al., 2015). In the final remodeling phase (8 week to 6 months), cellularity and matrix synthesis is reduced. The disorganized collagen is gradually resorbed and replaced by a more aligned and cross-linked collagen. Over the following months, continuous remodeling of the injured site leads

to a decrease in the area of the callus and improvement in mechanical strength. Nevertheless, complete regeneration of the tissue does not take place (Maffulli et al., 2002).

Growth factors are expressed during all stages of enthesis healing. They influence multiple pathways by signaling with different cell types in a complex manner to stimulate a response. Studies have sought to understand the temporal expression profile of the main growth factors involved during enthesis repair and their targets (Dahlgren et al., 2005; Kobayashi et al., 2006; Würgler-Hauri et al., 2007). Wurgler Hauri et al. studied the expression of BMP-12,13,14, b-FGF, CTGF, PDGF, TGF-β1, and COMP-1 in tendon-to-bone healing in a rat supraspinatus tendon insertion defect over a 16 week period (Würgler-Hauri et al., 2007). They showed that all growth factors were upregulated during the inflammatory stage, followed by a return to control or undetectable levels by 16 weeks. PDGF-B expression was detectable only at early time points at the insertion site and it coincides with early collagen I synthesis (Ojima et al., 2003) and activation of other growth factors such as TGF-β1 (Ojima et al., 2003; Porsch et al., 2014). Coinciding with the early upregulation of PDGF-B, the TGF-β1 expression was upregulated again at week 2, and independently at week 8. The delayed upregulation of TGF-β1 at week 8 can be correlated to the scarring process (Dahlgren et al., 2005; Galatz et al., 2006). BMP-12 was moderately expressed during all time points, with a significant increase around 8 weeks during the remodeling phase (Würgler-Hauri et al., 2007). BMP-12 has previously been reported to be expressed more in active fibroblasts (non-terminally differentiated, high-growth factor expressing) than in the resident tenocytes showing its active involvement in tissue remodeling (Fu et al., 2003; Wolfman et al., 1997). Thus, growth factors exhibit unique temporal expression profiles that correlate to specific stages in the enthesis repair process (Dahlgren et al., 2005; Würgler-Hauri et al., 2007). Although much information is known about the temporal expression of growth factors during rotator cuff healing, the mechanisms through which these growth factors influence healing are not completely understood. Such insight can be used to develop growth factor delivery devices that can spatio-temporally regulate therapeutic targets in rotator cuff repairs.

2.1. Mechanism of Scar Tissue Formation in Rotator Cuff Healing

The surgically repaired rotator cuff tendon insertion lacks the organized fibrocartilage structure of the native enthesis; instead, they heal via disorganized fibrovascular scar formation. TGF-β signaling plays an important role in pathological scarring across multiple organ systems (Penn et al., 2012) and has also been indicated in the formation of fibrovascular scar tissue in the rotator cuff (Liu et al., 2014). Of the three individual isoforms, TGF-β1 expression is positively correlated to scar tissue formation in adult tissue repairs (Wang et al., 2000). A gradual increase in TGF-β1 expression is seen in adult acute rotator cuff tears with a peak at day 10. In massive rotator cuff tears involving more than two tendons and a sub-scapular nerve damage, the scar tissue formation in the rotator cuff muscles was concomitant to the increase in TGF-β1 (Galatz et al., 2006). In another study, treatment of rotator cuff explants with TGF-β1 resulted in increased expression of alpha smooth muscle actin (α-SMA) characteristic of scar forming myofibroblasts (Premdas et al., 2001). On the other hand, fetal wounds heal without scarring, do not express TGF-β1, but TGF-β3 instead (Galatz et al., 2007). Thus, the levels of individual TGF-β isoforms can

significantly influence the process of scar tissue formation in rotator cuff tendon healing (Pryce et al., 2009).

The TGF-β1 isoform inflicts scar tissue formation via a number of mechanisms. The key cellular mechanisms involved in the scarring process are as follows: TGF-β1 induces differentiation of fibroblasts into myofibroblasts via expression of α-smooth muscle actin (Desmoulière, 1995). Myofibroblasts have been identified as the primary source of disorganized collagen III matrix synthesis in many fibrotic disorders (Todd et al., 2012). These myofibroblasts promote the induction of more TGF-β1 and other fibrogenic chemokines to the repair site (Zhang et al., 1995). TGF-β1 in turn ensures the survival of the differentiated myofibroblast population by preventing them from undergoing apoptosis (Zhang and Phan, 1999). The consequent increased callus size as a result of exaggerated matrix production and myofibroblast hyperplasia is further promoted by an inhibition of matrix metalloprotease activity (MMPs) (Farhat et al., 2015; Fenwick et al., 2008). Since an extensive discussion of the TGF-β mediated scarring phenomenon is beyond the scope of this review, we recommend the reader to the following comprehensive reviews on the subject: (Beanes et al., 2003; Penn et al., 2012).

3. Growth Factor Delivery

Growth factor delivery devices for rotator cuff repair include fibrous mats, braided constructs, sponges, and hydrogels made from synthetic polymers, extracellular matrix components, and inorganic materials. To meet the challenge of repairing a load bearing tissue, growth factor delivery devices are also designed to mechanically support the tissue during repair. These devices are superimposed on the tendon from the bursal-side in the sub-acromial space (Rodeo et al., 2007) or placed between the tendon and bone as an interpositional graft (Hee et al., 2011) and seek to promote the following targeted repair outcomes:

- **i.** The gap formed between the tendon and the bone is closed with healed tissue.
- **ii.** The collagen fibers in the newly formed fibrocartilaginous tissue insert and anchor into the bone.
- **iii.** The repaired tissue possesses the biomechanical properties necessary to support loading at the insertion site.

3.1. Design Characteristics of Growth Factor Delivery Devices

Regenerating the native structure of the rotator cuff enthesis would require recreation of the specialized structure and mechanical properties of this tissue. For effective regeneration, growth factor delivery devices for enthesis repair must fulfil following design criteria some of which have been reviewed elsewhere (Chainani and Little, 2016; Cheung et al., 2010):

- **i.** Support surgical handling, pliability, and suturability in mini-open and arthroscopic repairs.
- **ii.** Be sterilizable without affecting growth factor activity and mechanical properties of the scaffold.

iii. Maintain bioactivity of the growth factor through the shelf life.

iv. Be recumbent to the tendon-to-bone insertion with dimensions tailored to the sub-acromial space to prevent impingement.

v. Withstand the complex multiaxial loading of the tissue at the re-attachment of the tendon-to-bone through the duration of repair.

vi. Promote endogenous recruitment of stem and progenitor cells to augment tendon and fibrocartilage formation.

vii. Minimize fibro-vascular scar formation.

viii. Degrade slowly with non-toxic degradation products.

ix. Be resistant to infection.

Growth factors are incorporated in scaffolds via the following three main approaches: (1) physical adsorption onto scaffolds by simple dip coating (this method has poor control over the release rate with most of the growth factor being released within the first day (Uggen et al., 2010) and up to a period of 2 weeks (Tokunaga et al., 2015a), (2) encapsulation within polymeric or ceramic nano/micro-sphere scaffolds that can be injected or implanted at the defect site via incorporation inside hydrogels or scaffolds providing controlled release kinetics in the tissue microenvironment and enhanced growth factor protection (Lee et al., 2004; Yilgor et al., 2009), (3) Direct 3-D dispersion in a matrix or immobilization to a scaffold via electrostatic interactions or covalent bonding ensures controlled release and/or presentation of target growth factors (Park et al., 2009; Wang et al., 2017). Recently, supercritical fluid technology has been innovatively used to ensure three-dimensional encapsulation and controlled release of selected growth factors from a scaffold (Diaz-Gomez et al., 2016; Govoni et al., 2017). The reader is directed to the following excellent reviews for further reading on different growth factor delivery strategies (Santo et al., 2013a, 2013b; Wang et al., 2017).

3.2. Material Selection for Growth Factor Delivery Device

Direct delivery of growth factors by local injection at the site of injury has shown to promote tendon healing (Lamplot et al., 2014; Shah et al., 2013). Such delivery faces the limitations of growth factor loss via circulation and degradation, and need multiple injections that reduces patient compliance. The short half-life of growth factors reduce the efficiency of direct injection. To overcome these limitations, growth factor impregnated surgical sutures were applied (Dines et al., 2007b). These sutures are physician friendly, provide mechanical support, and can simultaneously deliver growth factors locally to the site of injury. However, burst release of growth factors within hours of coming in contact with body fluids and the limited loading capacity of sutures minimize their efficiency for rotator cuff enthesis repair that takes place over a period of weeks to months (Cummings et al., 2012; Fuchs et al., 2012). Therefore, the development of effective growth factor delivery devices that extend the delivery over the therapeutic repair window is of great interest.

Engineered synthetic biodegradable and natural extracellular matrix-based scaffolds loaded with growth factors have shown promise in regenerating the enthesis (Smith and Grande,

2015); (Peterson et al., 2015). Synthetic biodegradable polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), and polycaprolactone (PCL) have been used as scaffolds for mechanical reinforcement and growth factor delivery in rotator cuff repair (Chainani and Little, 2016; Derwin et al., 2009; Hakimi et al., 2013; MacGillivray et al., 2006). These polymers can be tailored to control the degradation properties, which can influence tissue- mechanics, infiltration, and remodeling, and enable controlled growth factor delivery. In an ACL reconstruction model, controlled release of bFGF from a poly-L-lactic acid (PLLA) scaffold enhanced ligament-like tissue formation and osseointegration, which compensated for the degradation and loss of mechanical support of the scaffold (Kimura et al., 2008). PLGA offers excellent pliability as well as the ability to tune the release rate by modifying the lactide/glycolide content and molecular weight (Babensee et al., 2000; Sahoo et al., 2010). However, PLGA exudes a majority of the growth factor payload via burst release within hours of implantation (Gombotz and Pettit, 1995). Furthermore, the accumulation of acidic polymeric breakdown products leads to synovial inflammation, denaturation and degradation of the growth factors encapsulated in the matrix, and increased matrix metalloprotease (MMP) activity. The combination of elevated acid levels and MMP activity can cause osteolysis of the cortical bone and failure of the repair tissue to integrate (Lipner et al., 2015). To overcome this limitation, Zhao et al. developed a PLGA electrospun core-sheath membrane loaded with b-FGF, which slowed down the burst release, and maintained b-FGF activity during the first 3 weeks *in vitro* (Zhao et al., 2014). This scaffold showed minimum inflammation and significantly improved collagen organization and ultimate load to failure of the healing enthesis in a rat rotator cuff chronic repair model. Although with a lesser elastic modulus than PLGA, PCL degrades slower and has greater failure strain, making it an excellent material for sustained delivery of growth factors in the poorly vascularized space of the rotator cuff (Lam et al., 2009; Woodruff and Hutmacher, 2010). Moreover, the hydrolytic degradation product of PCL, 6-hydroxylcaproic acid, has shown no osteolysis in long-term implantation studies in animal models (Woodruff and Hutmacher, 2010).

Natural biodegradable polymer chitin was assessed for scaffold augmentation in a rabbit model for rotator cuff insertion repair (Funakoshi et al., 2006). A 10 mm surgical defect was created at the humeral insertion of the infraspinatus tendon and the defect was covered with the chitin patch. Tendon-to-bone interphase covered with the chitin patch demonstrated greater cell number, better collagen fiber alignment, and greater mechanical strength than the unrepaired control. Although chitin faces batch-to-batch variability based on source it has been successfully applied for macromolecular delivery in other orthopedic applications (Jayakumar et al., 2011). We believe this material shows great potential in growth factor delivery for rotator cuff repair.

Extracellular matrix-derived materials, such as collagen, gelatin, heparin, and blood-derived fibrin, have been used to load growth factors for tendon-to-bone insertion repairs (Hee et al., 2011; Ide et al., 2009; Manning et al., 2011; Tokunaga et al., 2015a; Zhang et al., 2016). However, these polymers lack the mechanical properties necessary to support the complex loading at the rotator cuff insertions (Kosuge et al., 2013). To overcome this challenge, advanced composites that combine the defined mechanical properties of synthetic polymers with the bioactive cues from ECM proteins have been investigated (Leong et al., 2015;

Manning et al., 2011). A bioresorbable poly-4 hydroxy butyrate (P4HB) mesh embedded in a porous type I collagen sponge (Biofiber-CM) and loaded with fibroblast growth factor mimicking peptide (F2A) was tested in an ovine rotator cuff repair model (Peterson et al., 2015). P4HB is more pliable than traditional biodegradable polymers and the scaffold made from this material is easily inserted through an arthroscopic cannula. Its monofilament fibers resorb completely within a year, and has excellent biocompatibility (Martin and Williams, 2003). This scaffold-augmented repair showed improved tendon-like tissue coverage at the foot print, new bone formation at the interface, and lesser tears at the insertion on the humeral head (Peterson et al., 2015). Such combination strategies using existing FDA-approved materials to advance rotator cuff therapy can ensure faster translatability of promising materials.

3.3. Growth Factor Selection

Growth factors offer a simple and direct approach to improve healing outcomes in rotator cuff repair. Repair of rotator cuff entails the repair of the enthesis, which encompasses tendon, fibrocartilage, and bone. Interventions to repair such a complex tissue must factor in the heterogeneity of the tissue, and particularly the challenges posed by older patients, who have advanced degenerative tissue loss and diminished healing potential.

When a supraspinatus tendon tears, there is increased growth factor production at the injury site (Würgler-Hauri et al., 2007). The repair of the tendon-to-bone insertion involves growth factor-mediated migration of undifferentiated cells from the sub-acromial bursa and the underlying bone marrow (Koike Y, 2005 J Orthop Res). The growth factors involved include bone morphogenetic protein (BMP) 2,4,7; growth differentiation factor (GDF) 5,7; basic fibroblast growth factor (b-FGF); platelet-derived growth factor (PDGF); transforming growth factor (TGF)- β1-3; and connective tissue growth factor (CTGF). All these growth factors return to near physiological levels 16 weeks post-injury, establishing a connection between their *in vivo* upregulation and tissue repair (Chen et al., 2008; Würgler-Hauri et al., 2007). Despite this response by the body to heal, gaps often form between the tendon and bone after surgical repair of large rotator cuff tears (Reilly et al., 2004), which impair tendon structural and mechanical outcomes (Killian et al., 2014). Growth factors, being potent morphogens, can reduce the gap formation by promoting new tissue formation at the interphase by increasing cellular migration, proliferation, differentiation, and matrix synthesis (Oliva et al., 2011). In the following sections, we discuss interesting findings from studies applying growth factors to augment tendon-to-bone healing in animal models.

3.3.1. Bone Morphogenetic Protein-2, 3, and 7—Rotator cuff tendon tears result in unloading, and; therefore, loss of physical stimuli at the tendon-to-bone insertion. This unloading results in bone loss at the insertion site (Cadet et al., 2008; Waldorff et al., 2011). For formation of a mechanically functional enthesis, the tendon collagen fibers must be anchored into the bone via formation of a mineralized fibrocartilage (Dyment et al. 2015) and positive remodeling of the underlying epiphyseal bone. This understanding led to the use of osteoinductive factors such as BMP-2 through 7 for rotator cuff enthesis healing. Osteoinductive bone protein extract containing BMPs- 2 through 7, TGFβ- 1 through 3, and b-FGF was delivered though a collagen sponge in a sheep infraspinatus tendon detachment

model (Rodeo et al., 2007). New bone and soft tissue formation with increased load to failure was observed at 6 and 12 weeks following this treatment. However, when the data was normalized to tissue volume there was no difference between groups, suggesting that the growth factor treatment resulted in a poor-quality scar tissue. Perhaps the confounding limitations of gap formation and suture failure in the sheep model, along with the fast degradation and loss of growth factors from the collagen sponge used for delivery may have obscured the benefits of this growth factor cocktail.

BMP 2 and 7 have also been reported to induce chondrogenic differentiation (Dorman et al., 2012), and stimulate synthesis of fibrocartilage ECM components, proteoglycans and type II collagen (Flechtenmacher J. 1996, Kabuto et al 2015). In line with this, BMP-7 released from gelatin hydrogel sheets over a 3-week period post-operatively, significantly improved enthesis matrix production, and, as a result, tendon-to-bone maturing scores (Kabuto et al 2015).

In an indirect approach, BMP-2 was used to promote tendon-to-bone insertion healing by first injecting it into the flexor digitorum communis tendon in a rabbit hind limb to induce ectopic ossicle formation (Hashimoto et al., 2007). The resultant tendon/ossicle complex was surgically transferred onto the surface of the rabbit tibia to generate a stable tendon-to-bone junction. Failure loads were significantly higher in this treatment group compared to the control group without BMP-2 treatment. This shows that osteoinductive growth factors successfully promote enthesis healing when the engineered construct has pre-existing functionalities such as structural and mechanical compliance to the native tissue and biologically instructive ECM signals.

3.3.2. Growth Differentiation Factor GDFs 5, 6, and 7 (Bone Morphogenetic Proteins BMP 12, 13 and 14)—GDFs 5,6,7 are expressed in developing tendons and ligaments and are shown to promote tendon healing (Clark et al., 2001; Mikic et al., 2006). Within this family, GDF-7/BMP-12 shows greatest potential for tendon specific cell differentiation by expressing early phase tenogenic markers such as scleraxis (Scx) and tenomodulin (Tnmd) (Hoffmann et al., 2006; Shen et al., 2013). BMP-12 gene transfer in a complete tendon laceration model in the chicken resulted in a two-fold increase of tensile strength and stiffness of repaired tendons along with increase in collagen type I expression (Lou et al., 2001). Lamplot et al showed that direct delivery of adenovirus-mediated BMP 13 (AdBMP 13) into the rat supraspinatus tendon insertion defect demonstrated higher stress to failure than the delivery of platelet rich plasma that is routinely used in the clinic (Lamplot et al., 2014). This improvement in mechanical strength is positively correlated to the upregulation of tenocyte (tenascin and scleraxis) and ECM-associated (fibronectin and collagen type III and I) genes that are key in the early stages of tendon healing. Since the histopathological changes associated with rotator cuff tears are not localized only to the insertion but also in the macroscopic intact tendon portion (Longo et al., 2011), these growth factors that improve tendon morphology may be essential for functional healing of the rotator cuff.

In the developing tendon enthesis, GDF5/BMP-14 expressing progenitor cells proliferate and contribute to the linear growth of the tissue (Dyment et al., 2015). GDF5/BMP14 is also

associated with normal and pathological fibrocartilage differentiation during fracture healing in anatomically similar sites such as the intervertebral disc enthesis (Bostrom et al. 1995; Takae et al. 1999; Nakase et al. 2001). Therefore, this growth factor may be an interesting target to investigate for recapitulating developmental and injury-mediated processes in fibrocartilaginous tissues for the purpose of repair.

BMP-12 delivered in a type I/III collagen sponge improved tissue formation and mechanical properties in an ovine model compared to a hyaluronan paste carrier (Seeherman et al., 2008). The increased efficacy of BMP-12 when delivered through collagen sponge carriers may be because of its local retention at the repair site compared to the hyaluronan paste carrier. However, the healed tissue had a scar-like morphology and a higher cross-sectional area (Kovacevic and Rodeo, 2008). This fibrotic response may be due to the inhibition of MMP activity by GDFs (Enochson et al., 2014). Although increased MMP levels have been associated with tendinopathy and degenerative rotator cuff tears, and their inhibition shown improved collagen organization and fibrocartilage formation in acute rotator cuff tears (Bedi et al., 2010), global inhibition may disrupt later-stage remodeling of the repaired tissue..

3.3.3. Basic Fibroblasts Growth Factor (b-FGF/FGF-2)—Basic fibroblast growth factor (b-FGF) stimulates tendon fibroblast proliferation and migration (Chan et al., 1997) and induces differentiation of MSCs into tenocytes (Cai et al., 2013). Various models have suggested that the addition of b-FGF may increase the strength of the repair and accelerate tendon-to-bone remodeling (Ide et al., 2009; Peterson et al., 2015; Zhang et al., 2016; Zhao et al., 2014). In a rotator cuff supraspinatus injury model, b-FGF showed peak expression at day 7 (Würgler-Hauri et al., 2007). This early upregulation of FGF may promote gap closure between the tendon and the bone by increasing the proliferation of fibroblasts that synthesize collagen matrix.

More recently, FGF-2 has been used in rotator cuff tears because of anti-scarring properties. FGF-2 has been shown to block TGF-β1 mediated myofibroblast activation (Cushing et al., 2008) and induce apoptosis in the granulation tissue, thereby minimizing scar tissue formation (Akasaka et al., 2004). In line with these properties, decreased fibro-vascular scarring and improved biomechanical strength was seen within 6 weeks following FGF-2 delivery via gelatin hydrogels implanted as an interpositional graft between the injured supraspinatus tendon and bone (Tokunaga et al., 2015b). In another study, early delivery of FGF-2 from a fibrin sealant accelerated bone ingrowth and biomechanical strength at 2 weeks following acute rotator cuff repairs, but failed to show significant differences at later time points (Ide et al., 2009). This response may be because fibrin sealants release >50% of the payload within 24 hours of implantation (Ishii et al., 2007). In contrast, b-FGF released at a sustained rate over a 3 week period from a PLGA fibrous membrane significantly increased the collagen organization and failure load (Zhao et al., 2014). Prolonging the release of FGF may promote tissue formation and bone ingrowth at the early stage and controlled remodeling at a later stage.

3.3.4. Platelet Derive Growth Factor (PDGF)—PDGF is highly upregulated during the early phase of tendon healing (Kobayashi et al., 2006). This early upregulation of PDGF promotes the activation of other growth factors (Porsch et al., 2014) and stimulates cell

migration, proliferation, and matrix synthesis at the healing site (Lynch et al., 1989; Tsuzaki et al., 2000). In rat rotator cuff insertion tears, PDGF-BB, a homo-dimeric isoform released from gelatin hydrogel sheets, showed significantly higher cell proliferation, greater collagen fiber orientation, and ultimate load to failure at the insertion site (Tokunaga et al., 2015a). PDGF-BB delivered from collagen scaffolds enhanced cell proliferation and angiogenesis at the enthesis site during the early phase of healing (day 2), but failed to have any effect on fibrocartilage formation or collagen fiber maturity in the late phase (day 28) (Kovacevic et al., 2015). This may be because the accelerated diffusion of the PDGF from the collagen sponge leaves little to no growth factor during the later stages of repair *in vivo*; approximately 50% was released within the first hour *in vitro* (Bhargava et al., 2005). This begs the need for the development of sustained growth factor delivery devices to improve repair outcomes.

To promote sustained delivery of PDGF-BB in rotator cuff repairs, Min et al. developed a PCL/Pluronic F127 membrane that immobilized PDGF-BB via heparin (Min et al., 2016). This asymmetrically porous membrane facilitated sustained release of the growth factor and selective permeability (allowing permeation of oxygen/nutrients but preventing scar tissue invasion into defect region) thereby improving the repair of the fibrocartilaginous enthesis. The heparin-bound PDGF-BB immobilized on the membrane underwent sustained release over 42 days *in vitro*. However, this response may be altered *in vivo* as release of the growth factor from heparin would depend on a more complex dynamics involving multiple growth factor binding affinity and stability, cross receptor binding, and enzymatic and proteolytic degradation (Forsten-Williams et al., 2008). Nevertheless, these results support the efficacy of sustained PDGF delivery in regenerative healing.

3.3.5. Insulin-Like Growth Factor (IGF)—The IGFs have structural similarity to insulin, giving them the ability to bind to insulin receptors. IGF-1 improves functional outcomes of healing in rotator cuff tears (Dines et al., 2007a). In a chronic rotator cuff repair model, IGF-1 transfected tendon fibroblasts improved both toughness and maximal load to failure compared to untreated controls (Dines et al., 2007a). IGF-1 also acts in synergy with other growth factors such as PDGF-BB to increase cell proliferation and collagen production (Tsuzaki et al., 2000). Likewise, engineered ligament constructs treated with a combination of IGF-1 and TGF-β had higher maximal tensile load capacity compared to the ones treated with a single growth factor (Hagerty et al., 2012).

IGF-1 is known to prevent swelling and modulate inflammation in tendon and muscle injuries. The presence of IGF-1 in muscle regeneration is correlated with upregulation of inflammation-resolving macrophage M2; the conditional knock out of the IGF gene has also showed erratic healing response (Tonkin et al., 2015). Since inflammation and pro-inflammatory macrophages (M1) are exaggerated in tendon healing (Kawamura et al., 2005; Manning et al., 2014), growth factor therapies such as IGF-1 that can modulate macrophage polarization to promote inflammation resolution will be highly valuable in rotator cuff healing.

3.3.6. Transforming Growth Factor Beta (TGF-β)—TGF-β plays a critical role in pathological fibrosis in adult tissue repair (Samarakoon et al., 2013). TGF-β signaling,

involving isoforms 1 and 2, is highly upregulated during adult rotator cuff healing and this overexpression was concomitant to increased fibrosis in the rotator cuff muscles in massive tears (Liu et al., 2014). TGF-β1 significantly increases α-smooth muscle actin (α-SMA) in rotator cuff tears and has been suggested to contribute to retraction of the torn tendon (Premdas et al., 2001). Selective inhibition of TGF-β1 by a small molecule inhibitor SB431542 has shown significant reduction in fibrosis, fatty infiltration, and muscle atrophy in a murine rotator cuff tear model (Davies et al., 2016). This study demonstrates that TGF-β1 inhibition results in improved tissue quality in rotator cuff repair.

On the other hand, fetal wounds that heal without scar tissue show an upregulation of TGF-β3 isoform instead of 1 and 2 (Liu et al., 2014; Soo et al., 2003). The scar-free healing potential of TGF-β3 was tested by delivering it via an injectable calcium phosphate matrix in rat rotator cuff tears. However, the healing outcomes as measured by increased fibrocartilage area, improved collagen organization and bone formation were significantly better with the calcium phosphate matrix by itself than with TGF-β3 + calcium phosphate carrier group at the concentrations tested. Likewise, TGF-β3 delivery showed no reduction in scar tissue formation in a similar animal model (Manning et al., 2011). The positive effect of TGF-β3 in adult rotator cuff repairs are yet to be demonstrated.

3.3.7. Vascular Endothelial Growth Factor (VEGF)—Tendons are highly vascularized during development; however, this level of vascularization is not sustained as the tendon matures (Fenwick et al., 2002; Ferrara and Gerber, 2001; Peacock, 1959; Takasugi et al., 1978). Vascularization spikes after the inflammatory phase following injury of the mature tendon, with an increased expression of VEGF receptors on the endothelial cells inside the tendon (Pufe et al., 2005). Although, this vascularization leads to repair and remodeling of the injured tendon, it may also cause proteolysis of the ECM by the invading endothelial cells, thereby weakening the healing tendon (Savitskaya et al., 2011). Furthermore, VEGF expression and increased vessel density have been positively correlated with fatty infiltration (FI) and muscle atrophy (MA), two signature endpoints of degeneration (Lakemeier et al., 2010). In addition, VEGF was found to be responsible for the development of shoulder joint contracture: a condition in which the movements of the shoulder joint become markedly limited in patients undergoing rotator cuff repair (Handa et al., 2003). This could be because of motion pain or impingement in patients resulting from VEGF-induced increased synovial proliferation in the sub-acromial bursa (Handa et al., 2003).

Overall, it appears that the detriments of VEGF overweigh the benefits induced by this class of growth factor in rotator cuff healing. A thorough understanding of the association between vascularization and regeneration of the hypo-vascular rotator cuff tendon can better inform the application of this growth factor.

3.3.8. Platelet Rich Plasma—Individual growth factors may not provide conditions necessary for rotator cuff repair. Platelet rich plasma provides a rich source of growth factors and cytokines such as PDGF, TGF-β, FGF, VEGF, and IGF (Randelli et al., 2014). Although PRP therapy has many benefits in easy surgical implementation and patient safety, current

results are unclear regarding its benefit on structural healing or patient outcome of pain compared to surgery alone in rotator cuff repair (Randelli et al., 2015).

Platelet rich fibrin clot (PRFM), a variant of PRP with a fibrin matrix was tested in a randomized controlled trial on 79 patients undergoing arthroscopic rotator cuff repair. A suture anchor was placed in the tuberosity, the suture was passed through the PRFM and the tendon, with the PRFM secured between the tendon and bone at the interface. Tendon-to-bone insertion healing evaluated at 12 weeks post-operatively showed no difference between the PRFM and the control group. In a similar study using PRFM for augmentation of small and medium rotator cuff tear double row repair showed no significant differences in tendon thickness and coverage of greater tuberosity between PRFM and control group (Castricini et al., 2011). Based on these studies, the use of PRP for rotator cuff tendon has shown no benefit. This may be a result of variable PRP preparations used, undefined nature of PRP, as well as the level of platelet activation not being accounted for in each clinical study (Mazzocca et al., 2012). Further research with controlled PRP formulations are needed to assess accurately the benefits of this therapy.

3.4 Growth Factor Dose

Tailoring growth factor dose and availability to the coordinated tissue repair processes, spatio-temporally and safely, are critical factors limiting the use of growth factor delivery systems in the clinic. In pre-clinical models of rotator cuff repair, growth factors have been used in a broad range of concentrations; as presented in Table 1, PDGF-BB and FGF-2 have been used in a range of 0.5 to 5 μg and 0.5 to 50 μg per implant, respectively, in rat rotator cuff repair models. Likewise, as can be seen in Table 1, even within the same species, the dose difference is mediated by the type of model (acute or chronic repair); the placement of the scaffold (sutured on the bursal surface, or interpositional to the repaired tendon-to-bone insertion, or simply secured in a bony trough at the footprint); and the loading method (adsorbed, encapsulated, or presented in a controlled manner on the scaffold surface). High doses of growth factor can be detrimental to the tendon-to-bone healing. Improved tendon-to-bone interdigitation and biomechanical strength was observed with the application of 75 μg and 150 μg PDGF-BB incorporated in a type I collagen matrix, compared to 500 μg PDGF-BB, which was found to be detrimental to healing in an ovine rotator cuff repair augmentation model (Hee et al., 2011) (see Figure 3). Similarly, uncontrolled long-term presence of growth factors at the repair site can also led to poor tissue healing outcomes due to negative feedback at higher doses. For example, sustained expression of BMP-2 via adenoviral vector-based delivery lead to bone resorption and decreased mechanical properties of the healing tendon-to-bone insertion (Lipner et al., 2015). Controlled delivery of multiple growth factor doses that takes into consideration the spatiotemporal complexity of the injury microenvironment and acts in concert with the endogenous reparative processes via positive and negative feedback mechanisms will lead to significant improvements in growth factor therapy.

4. Innovative Materials for Rotator Cuff Repair

Recent innovations in material design and scaffold fabrication have led to engineered constructs that mimic the ultrastructural organization and mechanics of the native enthesis tissue (Chainani et al., 2013; Zhang, 2017; Zhang et al., 2012). One such strategy is to develop ECM-like nanofibrous structures by electrospinning and simultaneously create a gradient mineralized matrix (Lipner et al., 2014; Smith et al., 2012). Such functionally graded transitions can dissipate stress concentrations and toughen attachments (Lipner et al., 2014).

Gradients of biochemical cues on scaffolds have also been investigated. Polycaprolactone (PCL)/ Pluronic F127 membranes with counter gradients of dual growth factors - PDGF and BMP 2 released in a spatiotemporal manner for 35 days promoted ADSC differentiation into osteoblasts and tenocytes at the two ends of the gradients, respectively (Min et al., 2014). However, the authors did not evaluate the chondrogenic differentiation in the middle of the construct, which is most equitably stimulated by both PDGF and BMP2. Nevertheless, this construct shows promise for tendon-to-bone insertion repair by spatio-temporally regulating morphogen gradients.

Sustained release of a growth factor was achieved in a cellular environment by a heparin-fibrin based drug delivery system (Sakiyama-Elbert and Hubbell, 2000). This system includes an antithrombin III-based bi-domain peptide that is covalently cross-linked to a fibrin matrix by the transglutaminase activity of Factor XIIIa on the N-terminus. The C-terminal heparin-binding domain on the other end immobilizes heparin electrostatically to the matrix. The heparin in turn sequesters the growth factors by the interaction of its anionic sulphate groups with cationic amino acid groups found on growth factors (Thomopoulos et al., 2010, 2009, 2007). The release of growth factor from this matrix may occur by 3 mechanisms: (i) passive diffusion by dissociation of the growth factor from the matrix-bound heparin, (ii) active diffusion by proteolytic degradation of the peptide, and (iii) active diffusion by enzymatic degradation of the matrix (Sakiyama-Elbert and Hubbell, 2000). This heparin-fibrin hydrogel was then incorporated into multiple layers of electrospun PLGA nanofibers to provide structural integrity to the scaffold for surgical handling (Manning et al., 2013). This matrix has been shown to also retain and deliver mesenchymal stem cells with minimum toxicity in animal models (Gelberman et al., 2016). Innovations such as the fabrication of ECM-mimicking nanostructures, growth factor and mineral presenting gradients, etc. by integrating novel materials and fabrication technologies will lead the development of the next-generation scaffolds.

5. Novel Therapeutic Targets from Tendon and Developmental Biology

The early responding cells that migrate to the enthesis repair site may play a crucial role in adult tissue regeneration (Yoshida et al., 2016). These cells, which migrate from the bursal side of the tendon in response to supraspinatus tendon injury, express myofibroblast markers (Yoshida et al., 2016), and may be responsible for the scar-like tissue seen in the later stages of healing.

Recent studies have investigated the role of mechanoactive signals such as the hedgehog signaling in enthesis regeneration (Carbone et al., 2016; Dyment et al., 2015; Schwartz et al., 2015). Hedgehog responsive cells (Gli+) act transiently to promote mineralization of the neonatal healing enthesis, and its expression goes down after mineralization to prevent excessive remodeling of the tissue (Schwartz et al., 2015). On the other hand, the adult healing enthesis has a very small population of early-stage hedgehog responsive cells and shows incomplete mineralization (Schwartz et al., 2017). The failure to close the gap between the tendon and bone with repair tissue may prevent loading and thereby the activation of mechanoactive signals that are necessary for mineralization. These studies open up exciting avenues for using signaling molecules and growth factors in a time-dependent manner for rotator cuff enthesis regeneration. Manipulating time-sensitive signaling targets such as the hedgehog would require controlled and programmable advanced drug delivery strategies.

6. Dynamic Matrices for Endogenous Cell Recruitment in Enthesis Regeneration

Growth factor interactions with the ECM facilitate localized and spatially regulated signaling. Enhancing these interactions in clinically used growth factor applications can boost their therapeutic efficacy (Martino et al., 2014). A domain in placenta growth factor-2 (PIGF-$2_{123–144}$) binds exceptionally strongly and promiscuously to ECM proteins (Martino et al., 2014). By substituting the heparin-binding domain of growth factors such as VEGF, PDGF-BB, and BMP-2 with PIGF-$2_{123–144}$, engineered GF variants were generated that had super-affinity to the ECM. These ECM super-affinity variants induced repair in critical sized bone defects in rodent models. Enhanced cell homing by the controlled retention of these growth factor in a scaffold improved regeneration in these defects.

Growth factor binding to transmembrane protein receptors initiates cell signaling. Receptor binding of growth factors is regulated by interactions with heparan sulfate proteoglycans (HSPGs) that are ubiquitously present in the extra cellular matrix. Growth factor binding to HSPGs is closely regulated by the patterns of sulfate groups that are distributed along the length of the glycosaminoglycan (GAG) chains (Esko et al., 2009).The binding of the growth factors to HSPGs allow their local retention (Sarrazin et al., 2011), protection from degradation (Sadir et al., 2004), activation by oligomerization (Proudfoot et al., 2003), and presentation to cells (Handel et al., 2005).These varied sulfation patterns facilitate the formation of morphogen gradients that are essential for selective cell recruitment in the tissue regeneration process (Sarrazin et al., 2011). The next generation growth factor delivery strategies in enthesis regeneration will incorporate our emerging understanding of the HSPG-controlled growth factor interactions during scaffold design.

7. Animal Models of Rotator Cuff Repair

Animal models in rotator cuff rely heavily on the similarity of the anatomical macrostructures and functions to mimic the pathophysiology of rotator cuff tears (Lebaschi et al., 2016). Rat is the most commonly used animal model in rotator cuff repair assessments because of it’s anatomical and kinematic similarity to humans, with the acromial arch, a

supraspinatus tendon below the arch, and forward arm elevation. It is also cost effective. However, rat rotator cuff tears heal better with minimal fatty infiltration and have minimum post-operative tears unlike those seen in humans (Derwin et al., 2010b). To mimic fatty infiltration in the rotator cuff muscle as seen in humans, rodent rotator cuff tear models were created with simultaneous transection of the suprascapular nerve (Kim et al., 2012). Larger animals, in particular the sheep has been used to test the effect of new surgical scaffolds, and is an excellent model to study the chronic degenerative changes in the muscle following rotator cuff tear. However, the sheep lacks a coracoacromial arch and its weight bearing forelimb leads to failure of all repairs. The paucity of species-specific probes also limit the ability to conduct detailed histological analysis in this model. For more extensive literature on animal models in rotator cuff repair please refer to these excellent reviews (Deprés-Tremblay et al., 2016; Derwin et al., 2010b; Lebaschi et al., 2016).

8. Summary and Future Outlook

Rotator cuff tears are becoming increasingly common with more than half of the adults >65 years being affected. Retears after surgical repair are pushing for newer strategies in rotator cuff repair augmentation. Growth factor delivery from engineered scaffolds shows promise by enhancing the rate and quality of repair. Despite several growth factors showing improved healing in preclinical studies of rotator cuff repair, there are no growth factor formulations currently approved by the FDA for this indication. In fact, there are only two growth factor formulations currently approved by the FDA for clinical use in the Unites States mainly for the purpose of bone fracture healing: BMP2 (Infuse™, Medtronic Sofamor Danek, Inc.) and BMP7 (OP-1™, Stryker Biotech) (Santo et al., 2013b), both using Type I collagen as a carrier. To provide therapeutic benefit, supraphysiological doses of these growth factors have been used in the clinic. For example, the clinical doses of BMP-2 used in spinal fusion are associated with increased risk of cancer and ectopic bone formation (Carragee et al., 2013). The required excess of these growth factors may be explained by ineffective delivery systems that do not provide spatiotemporal regulation of the growth factor delivery. Overall, the safety, efficacy, and prohibitive cost of growth factor delivery limit their widespread clinical use.

Rotator cuff being a complex tissue, activation and mobilization of the heterogeneous cell population may require multiple complementary growth factor signals acting in concert. Therefore, finding the right combination of growth factors that can simultaneously promote tendon-enthesis tissue formation, mineralization, and integration of the repaired construct is of interest. Our evolving understanding of the role of growth factors during the different stages of healing will inform the design of sophisticated spatio-temporally controlled multiple growth factor delivery systems. These growth factor delivery systems must be designed to provide an active cell-instructive environment during the therapeutic healing period. Such a strategy would especially benefit the older population whose endogenous healing mechanism is affected by senescence and degeneration.

Cell-instructive environments are inherently present in natural ECM-based scaffolds. However, their use as growth factor delivery scaffolds is limited by their rapid degradation and uncontrolled release of growth factor in a short time period. Synthetic scaffolds can be

engineered with controlled degradation properties to deliver the growth factor payload as and when needed, while simultaneously being mechanically tailored to withstand the loading demands of the tissue over the remodeling period. Composites of these synthetic biodegradable scaffolds with extracellular matrix proteins would augment the repair by providing tailorable growth factor delivery and mechanical properties, while retaining the biological cues. Innovations in material design and scaffold fabrication have led to engineered constructs that mimic the ultrastructural organization and mechanics of the native enthesis tissue. Scaffolds presenting gradient biochemical cues and having sustained growth factor delivery capability have also been proposed. Our recent understanding of enthesis development and regeneration biology is pointing to newer targets for growth factor delivery strategies. We are entering an exciting area of growth factor therapy in rotator cuff repair. The next-generation growth factor delivery scaffolds must undergo comprehensive evaluation of host and foreign body response. Added capabilities such as surgical handling and amenability to arthroscopic repairs can expedite clinical translation of such scaffolds.

Acknowledgements

The authors gratefully acknowledge funding from Novartis, G600795, and the Office of the Vice President for Research, University of Connecticut, 401543-10301-20. Cato T. Laurencin, M.D., Ph.D. is a recipient of the NIH Director's Pioneer Award 2014, 1DP1AR068147-01.

Abbreviations

VEGF	vascular endothelial growth factor
BMP	bone morphogenetic protein
TGF-β	transforming growth factor beta
b-FGF	basic fibroblast growth factor
GDF	growth and differentiation factor
PDGF	platelet derived growth factor
MMP	matrix metalloprotease
COMP	cartilage oligomeric matrix protein
CTGF	connective tissue growth factor
IGF	insulin-like growth factor
α-SMA	alpha smooth muscle actin
SCX	scleraxis
SOX-9	SRY-box-9
PRP	platelet rich plasma
PRFM	platelet rich fiber matrix

IHH	Indian hedgehog
Gli^{+}	glioma associate oncogene
PlGF	placenta growth factor
HSPG	heparin sulfate proteoglycans
GAG	glycosaminoglycan

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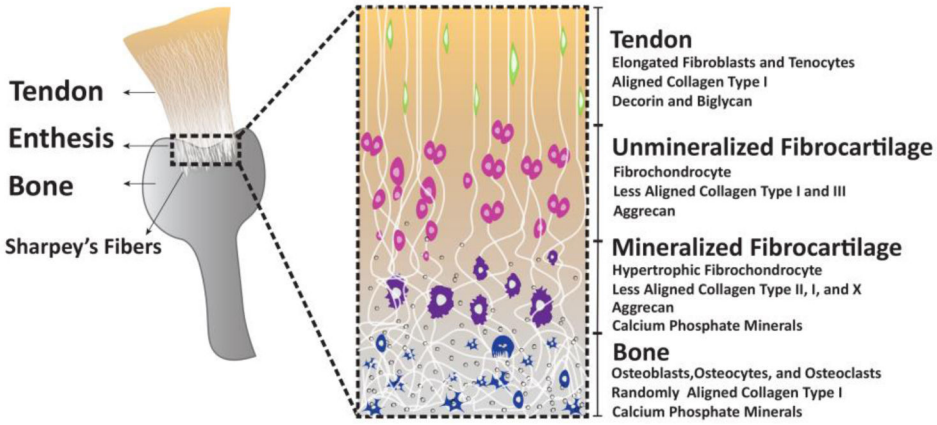

Figure 1. Structure and constitution of the native fibrocartilaginous enthesis.

Tendon anchors into the bone via a transitional fibrocartilaginous enthesis that constitutes tendon, fibrocartilage, unmineralized fibrocartilage, and bone. The transitions in cellular and ECM constitution are illustrated in the inset.

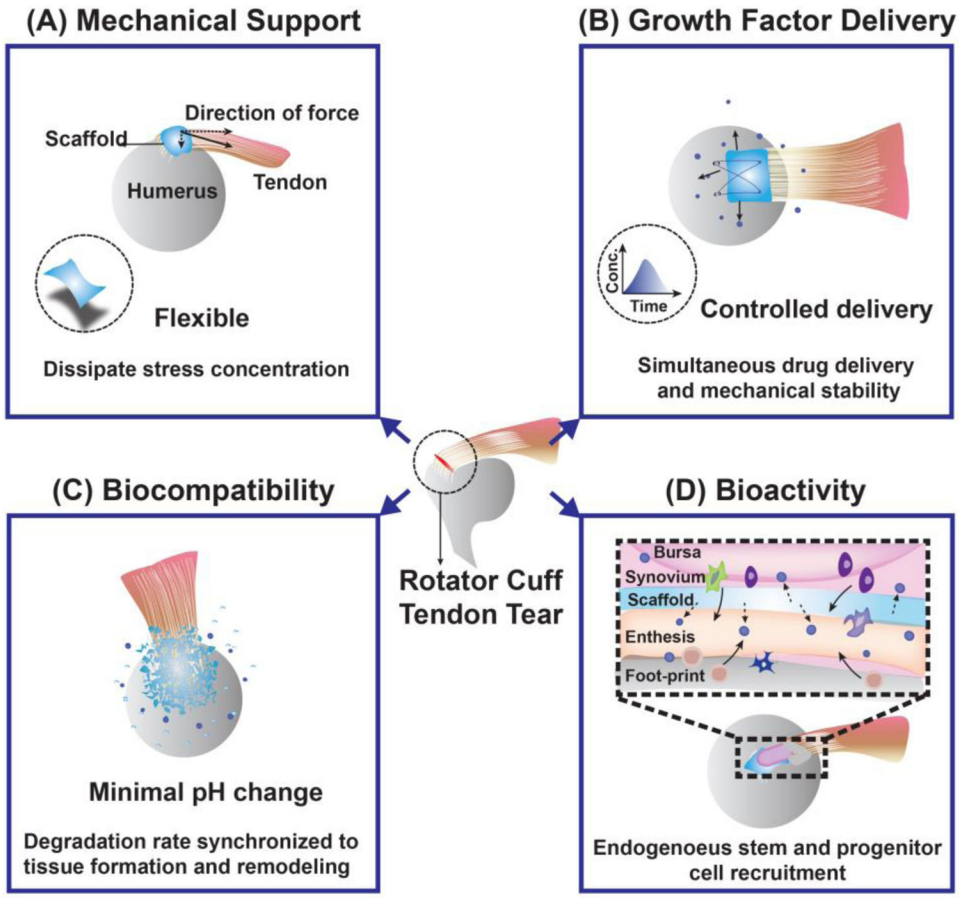

Figure 2. Desirable attributes of growth factor delivery scaffold for rotator cuff repair.

An example supraspinatus tendon-to-bone insertion tear at the humeral head is depicted in the center, with offsets (A), (B), (C) and (D) depicting the desirable traits of a growth factor delivery scaffold for this repair. Scaffolds must (A) mechanically support the repaired tissue and dissipate stress concentrations at the insertion; (B) deliver growth factor(s) in a controlled manner through the therapeutic time frame while being mechanically resilient to complex loading; (C) undergo degradation with minimal change in local pH with the degradation rate synchronized to the developing structure and function of the new tissue; (D) be cell-instructive to recruit stem and progenitor cells from the bursa and the underlying primary cartilage to the healing site to promote enthesis regeneration.

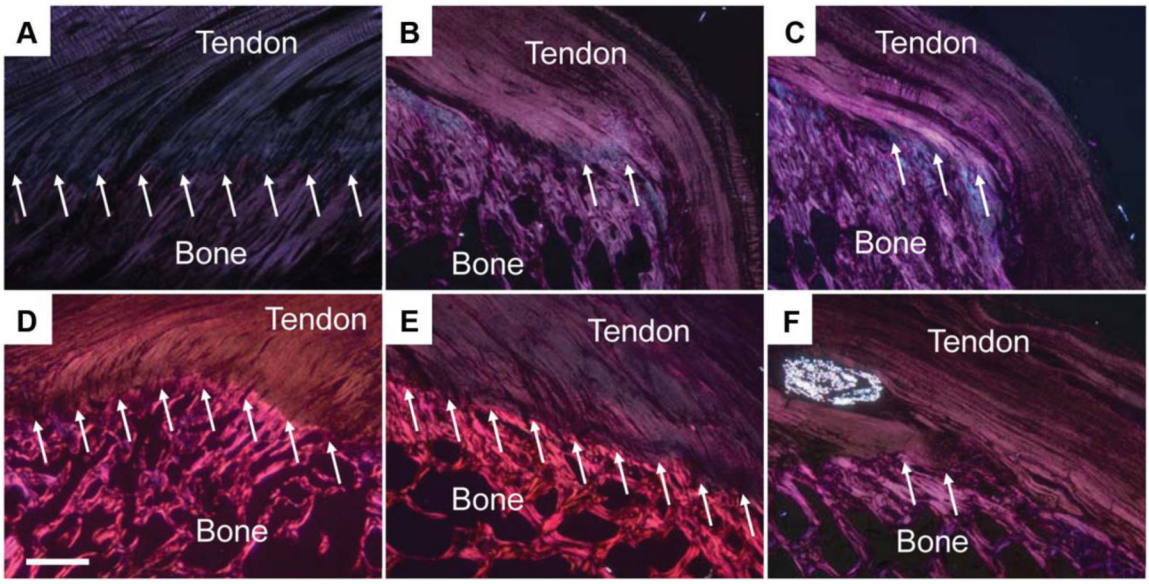

Figure 3. Polarized light images of hematoxylin and eosin–stained sections show growth factor dose-dependent changes in the tissue morphology (regions of interdigitation as noted by the arrows) at the repaired tendon-to-bone insertion site (Scale bar = 1 mm).

(A) Native tendon; (B) suture repair only; (C) collagen matrix+0 μg rhPDGF-BB; (D) collagen matrix+75 μg rhPDGF-BB; (E) collagen matrix+150 μg rhPDGF-BB; (F) collagen matrix+500 μg rhPDGF-BB. The morphological appearance was significantly improved in the 75 and 150 μg rhPDGF-BB augmented repairs when compared to the 0 and 500 μg rhPDGF-BB that showed poor morphology. Reprinted from Kee CH et al. Augmentation of a Rotator Cuff Suture Repair Using rhPDGF-BB and a Type I Bovine Collagen Matrix in an Ovine Model. The American Journal of Sports Medicine 2011; 39 (8):1630-39; with permission (SAGE publications).

Table 1.

Summary of key pre-clinical studies using growth factor delivery to augment rotator cuff repair

Growth Factor	Dose	Delivery Scaffold	Loading Method	Duration of Release	Animal Model	Scaffold Placement	Histological and Biomechanical Outcome	Reference
PDGF-BB	75, 150, 500 μg	Type I collagen matrix	Soaking		Sheep infraspinatus tendon detachment and acute repair	Interpositional to the repaired infraspinatus tendon-to-bone insertion	Better tendon-to-bone interdigitation and higher ultimate load-to-failure in the 75 μg and 150 μg PDGF-BB with type I collagen matrix group than the 500 μg group and suture only control at 12 weeks. Higher doses led to poor morphological appearance and biomechanics.	(Hee et al., 2011)
	0.5 μg	Gelatin hydrogel sheets	Soaking	100% released within 2 weeks during subcutaneous implantation	Rat supraspinatus tendon detachment and acute repair	Lateral to the repaired supraspinatus tendon-to-bone insertion	Better organized collagen fiber at the insertion site and significantly greater ultimate load-to-failure and stiffness in the PDGF-BB treated group than that in the suture repair and PBS-soaked gelatin hydrogel sheet controls at 12 weeks.	(Tokunaga et al.,2015a)
	0.6, 2, 6 μg	Type I collagen scaffold	Soaking		Rat supraspinatus tendon detachment and acute repair	Bursal to the repaired supraspinatus tendon-to-bone insertion	Dose-dependent response in cellular proliferation and angiogenesis in PDGF-BB delivery group at 5 days compared with repair only and scaffold only control. No significant difference between treatment and control groups on the area of fibrocartilage, collagen fiber orientation, or biomechanical properties at 28 days.	(Kovacevic et al., 2015)
	896 ng/cm	Fiberwire sutures	Dip coating	80% released within 1 hour, and remaining released in 48 hours	Sheep infraspinatus tendon detachment and chronic repair		Significantly better organized gap tissue with extensive cartilage formation at the tendon-to-bone interface in the rhPDGF-BB coated sutures repair group compared with controls at 6 weeks, but ultimate load-to-failure was equivalent to uncoated controls.	(Uggen et al., 2010)
FGF-2	0.5, 5 and 50 μg	Gelatin hydrogel sheet	Soaking	100% released within 2 weeks	Rat supraspinatus tendon detachment and acute repair	Interpositional to the repaired infraspinatus tendon-to-bone insertion	Significant improvement in mechanical strength in the FGF-2-treated group at 6 and 12 weeks. No significant difference between different doses.	(Tokunaga et al.,2015b)
	2 μg	Electrospun PLGA fibrous membrane	Emulsion encapsulation	50% released in 2 weeks in a sustained manner	Rat supraspinatus tendon detachment and chronic repair	Bursal to the repaired supraspinatus tendon-to-bone insertion	Significantly improved collagen organization and highest ultimate load-to-failure and stiffness in the bFGF–PLGA scaffolds than control suture repair and PLGA scaffold alone groups at 8 weeks.	(Zhao et al., 2014)
	3, 30 μg	Gelatin hydrogel sheet	Dip coating	30% released within 1 day and the remaining released in a sustained manner in approximately 2 weeks	Rabbit supraspinatus tendon detachment and acute repair	Placed in a created bony trough interpositional to the repaired infraspinatus tendon-to-bone insertion	Histologically and biomechanically enhanced rotator cuff tendon-to-bone healing in both 3 and 30 μg of FGF-2 impregnated gelatin hydrogel sheets with no significant difference between the two doses at 6 weeks.	(Tokunaga et al.,2017)

Growth Factor	Dose	Delivery Scaffold	Loading Method	Duration of Release	Animal Model	Scaffold Placement	Histological and Biomechanical Outcome	Reference
F2A (peptide mimetic of FGF-2)	1, 8 mg	Collagen-coated P4HB fibrous scaffold	Dripping		Sheep infraspinatus tendon detachment and acute repair	Bursal to the repaired infraspinatus tendon-to-bone insertion	The extent of delamination decreased with increasing doses of F2A, and more tendon-like tissue and new bone formation was observed in the growth factor augmented group at 8 weeks.	(Peterson et al.,2015)
BMP-2	50 μg/ml	Acellular dermal patch	Coated on the surfaces and then freeze dried	75% released in a sustained manner in 2 weeks	Rabbit supraspinatus tendon detachment and chronic repair	Interpositional to the repaired supraspinatus tendon-to-bone insertion	Greater new bone formation, cell penetration into the host bone, well connected interface with no gap formation, and significantly higher ultimate tensile load in the rhBMP-2 augmented repair group at 8 weeks.	(Lee et al., 2017)
BMP-7	0.5 μg	Gelatin hydrogel sheet	Coated on the gelatin hydrogel sheets	90% released in a sustained manner within 2 weeks	Rat supraspinatus tendon detachment and acute repair	Bursal to the repaired supraspinatus tendon-to-bone insertion	Significantly higher tendon maturing score and highest ultimate load-to-failure in the BMP-7 delivery from gelatin hydrogel sheet group than the following controls: direct injection of BMP-7, gelatin hydrogel sheet alone, and the suture repair alone, respectively, at 8 weeks.	(Kabuto et al., 2015)
BMP-12	50*/20 † μg	Type I collagen sponge	Soaking	90% released in a sustained manner within 2 weeks	Sheep infraspinatus tendon detachment and acute repair	* Interpositional to the repaired infraspinatus tendon-to-bone insertion	Higher collagen content, maximum tensile load 2.1 times greater in the rhBMP-12 delivered via Type I/III collagen sponge group than that of repairs treated with Type I/III collagen sponge alone at 8 weeks.	(Seeherman etal.,2008)
	75*/30 † μg	Type I/III collagen sponge				† Bursal to the repaired supraspinatus tendon-to-bone insertion		
TGF-β3	2.75 μg	Calcium phosphate matrix	Injected into the calcium phosphate matrix		Rat supraspinatus tendon detachment and acute repair	Placed in a created bony trough interpositional to the repaired infraspinatus tendon-to-bone insertion	Improved fibrocartilage formation and collagen organization at the enthesis in the calcium phosphate matrix alone group than the calcium phosphate matrix with TGF-β3 at 2 weeks.	(Kovacevic et al., 2011)
TGF-β1	0.1 μg	Gelatin hydrogel sheet	Soaking		Rat supraspinatus tendon detachment and acute repair	Interpositional to the repaired supraspinatus tendon-to-bone insertion	Tough fibrous tissues at the healing site with significantly higher ultimate load-to-failure and higher collagen content in the TGF-β1 gelatin hydrogel sheets group than saline control at 12 weeks.	(Arimura et al.,2017))