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Regenerative Engineering of the Rotator Cuff of the Shoulder

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Abstract

Rotator cuff tears often heal poorly, leading to re-tears after repair. This is in part attributed to the low proliferative ability of the resident cells (tendon fibroblasts and tendon-stem cells) upon injury to the rotator cuff tissue and the low vascularity of the tendon insertion. In addition, surgical outcomes of current techniques used in clinical settings are often suboptimal, leading to the formation of neo-tissue with poor biomechanics and structural characteristics, which results in re-tears. This has prompted interest in a new approach, which we term as “Regenerative Engineering”, for regenerating rotator cuff tendons. In the Regenerative Engineering paradigm, roles played by stem cells, scaffolds, growth factors/small molecules, the use of local physical forces, and morphogenesis interplayed with clinical surgery techniques may synchronously act, leading to synergistic effects and resulting in successful tissue regeneration. In this regard, various cell sources such as tendon fibroblasts and adult tissue-derived stem cells have been isolated, characterized, and investigated for regenerating rotator cuff tendons. Likewise, numerous scaffolds with varying architecture, geometry, and mechanical characteristics of biologic and synthetic origin have been developed. Furthermore, these scaffolds have been also fabricated with

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biochemical cues (growth factors and small molecules), facilitating tissue regeneration. In this Review, various strategies to regenerate rotator cuff tendons using stem cells, advanced materials, and factors in the setting of physical forces under the Regenerative Engineering paradigm are described.

Graphical Abstract

Keywords

rotator cuff regeneration; stem cells; synthetic and biologic scaffolds; growth factors; small molecules; mechanical stimulation; clinical trials

1. INTRODUCTION

In 2011, musculoskeletal diseases, disorders, and injuries resulted in expenditures of over \$175 billion (U.S. dollars).¹ Shoulder-related disorders affect significant portions of the population, and in particular the elderly, causing debilitating pain, reduced function, and joint instability.² Based on 2010 estimates, approximately five million people in the United States have some level of discomfort in the shoulder necessitating doctor's visits for further evaluation.³ Of the five million visits, approximately 400,000 patients underwent surgical procedures (Rotator Cuff repair) to treat rotator cuff lesions.⁴ Despite the high cost of surgical procedures (approximately \$15,000 per patient) and allied follow-up non-operative treatments (\$1800 per patient), high failure rates ranging between 20% and 70% have been reported after rotator cuff surgical repair.⁵ High failure rates are attributed to stiffness, infection, fatty infiltration, muscle atrophy, muscle retraction, and rotator cuff degeneration.⁵ Recent research suggests that the re-tear rates are highly dependent on the pre-operative tear size.⁶

According to American Academy of Orthopaedic Surgeons (AAOS), the treatment regimen for rotator cuff lesions can vary from administering non-steroidal anti-inflammatory drugs (NSAIDs), to physical therapy to surgical intervention.⁷ However, beyond a tear size of 3 cm, surgery is typically recommended to treat rotator cuff lesions. The techniques commonly used for rotator cuff repair include: open repair, arthroscopic repair, and mini-

open repair.⁷ Open repair and mini-open repair can provide transosseous fixation to replicate the native footprint, whereas the all-arthroscopic technique permits the preservation of deltoid bone, which is removed in the open-repair technique.⁴ In addition, arthroscopic debridement may be suitable for patients whose range of movements are limited and who require less rehabilitation and quick return to normal activities, compared to repair techniques.^{8,9} A recent meta-analysis of rotator cuff surgeries (all-arthroscopic vs mini-open) on patients with small, medium, and large tears showed no significant differences between the techniques in terms of surgery time, pain scores or range of motion (forward flexion and external rotation)¹⁰

To further improve surgical outcomes, surgeons sometimes augment the rotator cuff with allografts or xenografts to provide initial mechanical reinforcement, facilitate neo-tissue in-growth, and finally enable tissue remodeling. Over the past several decades, various such allo- and xenografts derived from human, porcine, bovine, equine, and ovine sources have been predominantly fabricated from dermal and subintestinal submucosa (SIS) tissues (Table 1).¹¹ In addition, several grafts have been also derived from pericardium, fascia lata, and forestomach tissues of animal origin (Table 1). More recently, scaffolds from absorbable (poly(L-lactic acid) (PLA) and poly(4-hydroxybutyrate) (P4HB)), partially absorbable (polycaprolactone-based polyurethane urea), and non-resorbable polymers (polyurethane) have been fabricated for mechanical augmentation to facilitate rotator cuff regeneration (Table 1).

Despite augmenting with extracellular matrix (ECM) devices, significant improvements in terms of healing mechanics have not yet been realized, evidenced by continued failure rates.¹² Therefore, a current focus has been to identify strategies that can result in achieving higher success rates in rotator cuff tendon healing. Several possible avenues such as stem cell technologies (scaffold, and scaffold-free approaches), growth factors (GFs), other biologics such as platelet-rich plasma (PRP), in conjunction with existing techniques, are being actively pursued for rotator cuff regeneration.

Mimicking the native tissues structure and architecture in scaffolds is one approach to provide geometrical cues to facilitate neo-tissue in-growth. By the incorporation of GFs and small signaling molecules, multiple signaling pathways^{13–19} involved in rotator cuff regeneration can be activated. Another potential therapeutic approach is via the use of stem cells/progenitor cells, instead of tenocytes, muscle satellite cells, mesangioblasts, and pericytes, for rotator cuff regeneration. The advantages of using stem/progenitor cells include their proliferative and differentiation capabilities into cell lineages corresponding to the tissue type, enabling appropriate ECM secretion with advanced temporal and spatial precision.²⁰ Therefore, it is conceivable that a convergent approach encompassing salient features from distinct fields is needed for successful tissue regeneration. By converging advanced materials science, stem cell science, physics and developmental biology, a new paradigm that we have termed “Regenerative Engineering” can be used to regenerate damaged complex tissues. Here we describe efforts to utilize Regenerative Engineering techniques, including the use of advanced scaffolds, cell sources, and other biologics such as GFs and PRPs, that have shown promise in rotator cuff regeneration.

2. ROTATOR CUFF TENDON BIOLOGY AND BIOMECHANICS

The muscles of the rotator cuff consist of the supraspinatus, infraspinatus, teres minor, and subscapularis, which amalgamates into one continuous tendon structure (rotator cuff tendons) and attach onto the lateral aspect of the humeral head.²¹ The merging process occurs in a controlled fashion with the supraspinatus and infraspinatus muscles binding ~1.5 cm before the insertion site, followed by infraspinatus and teres minor near the myotendinous junction.^{22,23} The amalgamated structure then binds with surrounding structures to form a capsule–cuff complex. The amalgamated tendons along with the surrounding structures act together in transmitting force from muscle to the bone.²² The surrounding structures also consists of an epitenon (Figure 1A), a loose connective tissue covering the tendon tissue by a sheath-like layer providing vasculatures via neural system.²⁴ Likewise, endotenon (Figure 1A), a thin reticular connective tissue, covers several epitenons, providing the vasculature for maintaining the cell population of the tendon tissue by homeostasis.²⁴

Biochemically, the musculotendinous unit is comprised of water (70%) and collagens (30%). By wet mass, tendons are chiefly type-I collagen with minor concentrations of other collagens (types II, III & VI, IX, X, and XII) and elastins.²⁵ The collagens are synthesized by the tendon fibroblasts/tenocytes, forming the basic structural unit of the tendon.²⁴ The characteristic triple-helical structure of collagens is achieved by the self-aggregation of the collagen precursor, procollagen, resulting in collagen fibrils (Figure 1A).²⁶ Each collagen fibril repeats that are ~70 nm bundle together forming collagen fibers (100–500 μm).²⁷ The cross-linking of the collagen fibrils is achieved by the enzymatic (for example, lysyl oxidase)²⁸ and non-enzymatic routes (reduction of sugars), in the presence of copper and manganese,²⁴ resulting in significant increases in the mechanical properties (modulus, stiffness, and reduction in elongation). The presence of collagens and proteoglycans (PGs) influence the physical and chemical properties and remodeling characteristics of the tendon tissue. For example, type-III collagen facilitates the fibril formation and extensibility of tendon tissue. However, the synthesized type-III collagens with overall smaller fiber diameters have poor mechanical properties. The primary role of type-III collagen is to act as temporary scaffold, for the future synthesis of type-I collagen fibrils.²⁹ Additionally, type-III plays a key role in providing crimp-like structure, which facilitates elongation of tendon tissue (1–3%).²⁵ By acting as a buffer, elongation of the tendon tissue prevents rupture from sudden loading on to the tissue. Likewise, type-XII collagen influences the homeostasis of type-I collagen, by interrupting the triple helical structure, and subsequently stimulating the regeneration of uninterrupted type-I collagen fibrils.³⁰

The ECM contains also elastins, and proteoglycans (PGs) such as decorin, fibromodulin, biglycan (BG), lumican, and aggrecan (ACAN), which play a key role in the fibrillar assembly and growth of the tendon microstructure by binding with collagens.³¹ Being soluble in water and therefore capable of hydrating readily, PGs provide resistance against compressive forces to the tissue.³² In addition, PGs binds with proteins, GFs, and cytokines, facilitating activation and regulation of several signaling cascades implicated in the homeostasis and remodeling of the tendon tissue.^{33,34} For example, decorin is well known to bind with transforming growth factor- β (TGF- β) and epidermal growth

factor (EGF).^{35,36} Likewise, BG, fibromodulin, and lumican have been reported to bind with type-I collagen. Similarly, binding of aggrecan and versican with hyaluronan, a key component in cartilage tissue, with increased levels having been observed in regions that undergo compression.³⁶ Other well-known PGs that bind with tendon-related GFs (discussed later) include heparin sulfate with fibroblast growth factor (FGF- β)³⁷ and granulocyte-macrophage colony stimulating growth factor (G-CSF);³⁸ chondroitin sulfate (CS) with platelet factor-4;³⁹ and heparin with vascular endothelial growth factor (VEGF).⁴⁰

Tendon fibroblasts and tenocytes (mature fibroblasts) are the predominant cells (~95%) present in the tendon tissue.²⁵ Other cells (~5%) present in the tendon tissue include chondrocytes, vascular, and synovial cells.²⁵ Tendon fibroblasts are observed in the proper tendon region that undergoes tension, whereas chondrocytes are observed in the mineralized and non-mineralized regions that undergo compression.⁴¹ Likewise, vascular and synovial cells are found in regions that contain vasculatures and synovial sheath (epitenon and endotenon) in the tendon tissue.^{24,41} The fibroblasts with lengths and widths ranging from 20 to 70 μm and from 8 to 20 μm , respectively, are typically arranged in parallel along the collagen fibers.²⁴ Unlike other fibroblasts, however, the shape of the tendon fibroblasts varies considerably from ovoid to long spindle-shaped cells.²⁴ The primary role of tendon fibroblasts residing in epitenon and endotenon is to initiate the healing process by migrating and thereafter proliferating in the wound site. In addition, tendon fibroblasts synthesize various collagens and PGs.²⁹ The secondary role of the tendon fibroblasts is the regulation of tendon ECM proteins such as fibronectin (FN), thrombospondin, undulin, and tenascin-c (TN-C).⁴² TN-C is a key glycoprotein implicated in tendon biology, due to its ability in providing mechanical stability to the ECM.^{43–45} In addition, fibroblasts also regulate N-cadherin and vinculin expression, which are implicated in muscle remodeling and cell proliferation, respectively.⁴⁶ In addition to fibroblasts, tendon tissue has progenitors/stem cells that have been identified, isolated, and characterized for their potential to differentiate into various mesogenic lineages (discussed later).⁴⁷

Rotator cuff tendons can be classified into four zones based on their proximity to the bone and muscle insertion sites.⁴⁸ Zone I is defined as the proper tendon with ligament/tendon like characteristics with type-I collagen being the predominant collagen in the ECM. Likewise, zone II is classified as non-mineralized fibrocartilage comprised of collagens type-II and -III. Similarly, zones III and IV are classified as mineralized fibrocartilage and bone, respectively, with their respective collagen being type-X and -I, respectively.⁴⁹ In addition to the collagen types, the collagen fiber orientation also varies from parallel arrangement (in tendon) to random arrangement (in bony site) (Figure 1B). The gradual transition in collagen fiber orientation also minimizes the stress concentration and aid in the load transfer between the bone and tendon.⁵⁰ Like collagen types, the ECM composition also varies throughout the zones: for example, from decorin (in zone I) to mineral components (in zone IV).⁵⁰

The presence of various collagens, PGs, and mineral contents causes non-linearity, heterogeneity, and anisotropy in the mechanical behavior of the tendon tissue.²⁵ All ECM components (collagens, PGs, elastins, etc.) and their corresponding microstructure (orientation, arrangement, and cross-link density) provide viscoelasticity to the tendon tissue.⁵¹ The typical stress–strain curve of a tendon tissue shows three distinct regions

leading to ultimate rupture of the tissue (Figure 1C). In the first region, upon loading, 1–4% strain is evidenced without concomitant increase in the stress values.

This is due to the uncrimping of the tendon tissue by straightening of the collagen fibrils. The extent of uncrimping depends on the crimp angle, orientation, and proximal location (to bone and tendon).⁵³ Beyond this strain value, a linear region obeying Hookean law is evidenced in which elastic stretching of collagen fibrils takes place. The extent of the linear region determines the modulus (slope of plot) and stiffness of the tendon tissue.⁵⁴ Beyond this region, the tendon tissue fails by macroscopic rupture of the collagen fibrils. Collagen-mineral interactions,⁴⁹ collagen architecture and their distribution⁵⁵ ultimately determine the biomechanical behavior of the tendon tissue. For instance, tendon tissues containing higher initial fiber distribution and alignment have demonstrated higher stiffness and modulus values.⁵⁵

Several molecules such as type-XII collagen, TN-C, and elastins, in addition to type-I collagen, actively partake in stabilizing the tissue. For example, upon loading, type-XII collagen acts by disrupting the interactions between the type-I collagen fibrils, thereby allowing them to glide past each other. Similarly, animals lacking decorin or fibromodulin genes have shown abnormal fibrillar structure with low modulus and stiffness.^{56,57} Besides these biochemical aspects, material properties such as creep, stress–relaxation, and hysteresis,⁵⁸ and loading conditions (strain rate),⁵⁹ also play a key role in determining the mechanical behavior of the tissue.

3. CELL SOURCES FOR ROTATOR CUFF TENDONS HEALING

The use of stem cells for regenerating soft musculoskeletal tissues is very attractive for several reasons. The first and foremost is their innate capability to modulate the inflammation and immune response by secreting various paracrine factors such as GFs (including angiogenic factors) thereby having direct effect on various immune cells.⁶⁰ In addition, stem cells have the ability, irrespective of their origin (hematopoietic or non-hematopoietic), to undergo both symmetric and asymmetric divisions.^{60,61} Finally, stem cells are capable of differentiating into various tissue phenotypes such as those found in complex soft tissues.⁶² Due to the complexity of the tissue, it can be envisaged that utilizing native resident cell types (for example, tenocytes) would not be sufficient to regenerate rotator cuff tendons. Therefore, alternatives such as stem cells, having intrinsic capabilities to differentiate into complex cell phenotypes are being actively studied for regenerating complex soft tissues.

3.1. Bone Marrow-derived Mesenchymal Stem Cells (BMSCs).

BMSCs are the most widely studied stem cell source for soft tissue regeneration, including rotator cuff tendons. One reason for their wide use is their extensive history as evidenced by large volumes of *in vitro* and *in vivo* studies. Initially demonstrated by Friedenstein and co-workers for their capability to undergo osteogenic differentiation,⁶³ later studies have shown the capability of MSCs to undergo chondrogenic, adipogenic, myogenic, neurogenic, and tenogenic/ligamentogenic lineages,⁶⁴ triggering interests in their use for musculoskeletal

applications. The use of BMSCs for regenerating soft musculoskeletal tissue, including rotator cuff repair, began extensively in the past decade.

To facilitate clinical translation, rapid processing of tissues in clinical settings is vital, so that processed cells can be administered during surgical procedures for treating rotator cuff lesions. In this regard, concentrated bone marrow aspirate (cBMA) has been isolated and administered in a single step during the rotator cuff reconstruction procedure.⁶⁵ To further enhance the prospects of MSCs in successful rotator cuff regeneration, various strategies such as bone marrow stimulation, application of GFs, gene transfections, co-culture systems with resident cells, intra-articular and intra-muscular injections, have been developed and studied.

The microfracture procedure is commonly employed in repairing/regenerating cartilage tissue, by recruiting/simulating bone marrow stem cells into the injured site by a process called bone marrow stimulation (BMS). Likewise, BMS has also been used for recruiting bone marrow progenitors into the lesion site. One such study in a full thickness articular cartilage injury (shoulder) in humans demonstrated an 80% success rate with decrease in mean pain scores from 3.8 to 1.6. Also, the patients had a satisfaction rate of approximately 80%, with greatest improvements seen in patients who had microfracture in humeral lesions.⁶⁶ The key drawbacks of this study included the lack of biomechanical analyses and analyses of the quality of the tissue formed. To study the cell fate and its subsequent effects on healing, Kida et al. utilized green fluorescent protein expressing (GFP) rats wherein one shoulder was subjected to additional drilling to facilitate BMS before suturing. Histologic analyses at 4 and 8 weeks, showed the presence of large numbers of fibroblast-like cells and extensive matrix secretion in the drilled group, compared to the controls (no drilling). Finally, biomechanical analyses at 8 weeks showed significant improvements in the ultimate force to failure in the drilled group, thereby demonstrating the potential of BMS in treating rotator cuff lesions.⁶⁷

Subsequently, Taniguchi et al. showed the efficacy of bone marrow simulation (BMS) at the rotator cuff footprint facilitating cuff integrity in large massive tear in 111 patients. The BMS group showed a lower percent re-tear rate in the type IV–V category, compared to the non-BMS group (higher percent of type IV–V re-tear rates).⁶⁸ Similarly, Jo et al. reported low re-tear rates (22%) in 57 patients, who underwent additional drilling procedure, compared to 67 patients (45% re-tear rate) who underwent an arthroscopic rotator cuff repair procedure alone. Although higher cellularity was seen via fluorescent-activated cell sorting (FACS) analyses in the BMS group, its effects on structural remodeling and integrity (9-month time point) were not observed.⁶⁹ Due to the lack of bone tunnels and therefore low BMSC cell numbers in the vicinity of the tendon tissue, advancements in BMS technology through the tuberosity is hampered for rotator cuff augmentation (Figure 2A).^{70,71} One way to overcome this drawback is by the use of biological factors such as granulocyte-colony stimulating factor (G-CSF), which has shown to mobilize hematopoietic stem cells (HSCs).⁷² In a rotator cuff injury model, G-CSF administration demonstrated higher composite scores for cellularity at 12 and 19 days.⁷³ A study by Ficklscherer et al. showed enhancements in the healing process in the presence of G-CSFs evidenced by biomechanical and histological outcomes.⁷⁴

BMSCs are frequently administered in conjunction with other biologics (such as PRP) to support rotator cuff repair. Likewise, BMSCs are also delivered by 3-D scaffolds to promote viability, proliferation, and differentiation of BMSCs inside the surgical site.⁷⁵ BMSC administration (in fibrin sealant) was evaluated with respect to collagen fiber organization, areas of metachromasia, and proteoglycan secretion in the neo-tissue. Collagen birefringence experiments showed similar fiber organization across the study groups (fibrin, fibrin/BMSCs or none).⁷⁵ In addition, new cartilage formation was observed only at the early time point and not at later time points.⁷⁵ Histologic and biomechanical evaluations showed no significant improvements, demonstrating simple administration of autologous BMSCs (1 million cells per animal) to be insufficient for repairing/regenerating rotator cuff lesions in their study.⁷⁶

Similar effects were also observed by Degen et al. who studied cartilage formation by administering human BMSCs in an athymic rat rotator cuff model.⁷⁷ At the 2 week time point, Safranin-O staining showed significant increases in fibrocartilage formation (~19%) compared to the controls (~9.2%), whereas at the 4-week time point, no such statistical significance was observed.⁷⁷ The same trend was also seen in coherency (collagen fibers), energy absorption (collagen fibers), and biomechanics, all of which showed no statistical significance between the groups at 4 weeks.⁷⁷

Even though these two studies reported no significant long-term effects of BMSCs in the biochemical composition or biomechanical behavior of the formed neo-tissue, several animal models as well as pilot human studies have shown significant improvements in the final outcomes (biochemical, biomechanical, or motor behavior analyses). For example, a study evaluating the BMSC administration in bone-tendon junctions in a rat model reported significant improvements in the overall healing rates in the MSC group (>69%), compared to the control (~40%).⁷⁸ Likewise, the biomechanical evaluation demonstrated statistical significance between the study groups (MSC vs chondrocytes vs suturing) at all time points (15, 30, and 45 days).⁷⁸ Moreover, immunohistochemical evaluation demonstrated regeneration of enthesis (zones II and III), similar to that of native tissue, which was largely absent in the other study groups (suturing and chondrocytes) (Figure 2B).⁷⁹ A subsequent controlled pilot study on 45 patients further showed improvements in the healing with concomitant decreases in re-tears at both short (3 and 6 months) and long time points (1, 2, and 10 years), with high tendon integrity (87%) after autologous BMSC administration ($51,000 \pm 25,000$), compared to the controls that showed low tendon integrity (44%).⁸⁰

Likewise, another pilot study (12-months) evaluating magnetic resonance imaging (MRI) and University of California, Los Angeles (UCLA) score outcomes of 14 patients administered with autologous mononuclear stem cells (3.81×10^8) showed excellent tendon integrity in all cases with six patients showing formation of high signal intensity at the critical zone (enthesis).⁸¹ Functional evaluation upon BMSC administration (4.7×10^8 cells) in 102 patients showed dramatic reductions in disabilities of the arm, shoulder, and hand (DASH) scores (36.1 to 17.1 after administration) and numerical pain scales (NPS) (4.3 to 2.4 after administration), indicating the potential of the approach for treating rotator cuff lesions.⁸²

The effects of mitogenic GFs such as fibroblast growth factor, bFGF, platelet-derived growth factor (PDGF-BB), and differentiation factors such as insulin-like growth factor-1 (IGF-1), growth and differentiation factor-5 (GDF-5), and bone morphogenetic protein-12 (BMP-12) on BMSCs are well known and have been extensively studied.^{48,83,84} Recently, insulin, which closely resembles IGF-1 was reported as a molecule of interest for differentiating BMSCs into tendon. After identifying optimal dosages of insulin (10^{-10} mol/L), via real time polymerase chain reaction (RT-PCR), Western blotting, and immunocytochemical analyses, the authors demonstrated the significant up-regulation of the tendon-related proteins/genes collagen type-I (COL1A1), collagen type-III (COL3A1), scleraxis (Scx), TN-C, decorin, similar to those levels observed in IGF-1, bFGF and GDF-5 administration, demonstrating the potential of insulin in rotator cuff generation (Figure 2C–G).⁸⁵ Similarly, PRP continues to gain interest for tendon regeneration as they are theoretically capable of releasing various GFs such as VEGF, FGF- β , and TGF- β . Several formulations (discussed later) based on PRP therapy are currently being investigated for rotator cuff regeneration.

For example, when a BMAC-PRP combination was cocultured with tendon-derived stem cells (TDSCs), the population doubling time decreased slightly from 48.4 ± 1.4 to 46.6 ± 1.9 h.⁸⁶ More importantly, a clinical study demonstrated significant reductions in the visual analog scale (VAS) score from 5.8 ± 1.9 to 2.8 ± 2.3 at 3 months, with the administration of BMAC-PRP. Similarly, ultrasound studies revealed decreases in the torn area size from 30.2 ± 24.5 to 22.5 ± 18.9 mm², indicating the multi-potent effects of PRP and BMSCs in rotator cuff regeneration.⁸⁶

While PRP, specific GFs, and signaling molecules provide more generalized direction of BMSCs toward specific lineages; encoding specific transcription factors in MSCs is an alternative option for more effective tissue specific differentiation. For example, BMSCs overexpressing Scx, a transcription factor responsible for tendon and enthesis development have been used for rotator cuff regeneration.⁸⁷ Likewise, the use of tendon derived stem cells (discussed later) encoding for tumor necrosis factor alpha-stimulated gene/protein 6 (TSG-6), a binding enhancer of hyaluronan (glycosaminoglycan) to the cell surface receptor CD44,^{88,89} and zinc finger transcription factor early growth response 1 (EGR1), a transcription factor associated with tendon marker Scx,^{90,91} have been shown to improve the mechanical strength as well as bone–tendon healing in rat and rabbit rotator cuff models, respectively.^{92,93} Conversely, silencing the expression of factors such as TGIF1, a transcription factor observed in very low levels during chondrogenesis, can result in successful enthesis formation.⁹⁴ Despite these encouraging results, other studies have also reported the less than optimal performance of genetically modified cells. For example, bone morphogenetic protein-13 (BMP-13), a GF implicated in tendon and cartilage repair, did not show improvement in cartilage formation (602.5 ± 249.0 vs 596.5 ± 201.2 mm² for non-transfected MSCs), and mean load to failure (11.2 ± 2.5 and 11.2 ± 2.3 N for non-transfected MSCs), indicating the need for further experiments in utilizing gene-modified MSCs for rotator cuff regeneration.⁹⁵

3.2. Adipose-Derived Mesenchymal Stem Cells.

Like BMSCs, adipose-derived MSCs (ADSCs) are capable of differentiating into various musculoskeletal lineages such as adipogenic, chondrogenic, osteogenic, myogenic, and tenogenic lineages.⁹⁶ In addition, ADSCs secrete various GFs and cytokines by paracrine mechanisms, and impart their influence on various immune cells (T-cells, natural killer cells, dendritic cells).^{60,97} However, unlike BMSCs, large cell numbers (several thousand folds higher) can be obtained from a minimally invasive liposuction or lipoplasty procedure, making ADSCs more attractive for many investigators, for musculoskeletal regeneration, including rotator cuff repair.^{60,98} Like BMSCs, the final outcomes in terms of biochemical composition, biomechanics, histologic, immunohistomorphometric analyses, have been reported to range from very successful to no significant improvements in rotator cuff function.

A study by Mora et al. evaluated the effects of ADSCs on the biomechanical and histologic properties of the regenerated rotator cuff in a rat model.⁹⁹ Marginal statistical significance in absorbed energy and mechanical deformation were seen between ADSC and control groups at 2 weeks, which diminished at later time points (4 weeks).¹⁰⁰ However, Oh et al. demonstrated the generation of large compound muscle action and low fatty degeneration in an ADSC-administered group using a rabbit model.¹⁰¹ Besides ADSCs, intra-muscular (IM) injections of stromal vascular fraction stem cells (mixture of endothelial progenitors and ADSCs) have also shown reduced fibrosis and improved muscle quality, hallmarks of proper bone-tendon healing.¹⁰²

Chen et al.¹⁰³ and Uysal et al.¹⁰⁴ have reported significant increases in the load to failure values of ADSC-injected groups, either alone¹⁰³ or in conjunction with PRP or GFs.¹⁰⁴ For example, Chen et al. reported load to failure values of 15.87 ± 2.20 N in an ADSC-administered group compared to the control group (11.20 ± 1.35 N) after 7 days of ADSC injection.¹⁰³

At 28 days, biomechanical results of the ADSC group closely matched the load to failure values of uninjured native tissue, demonstrating the restoration of injured rotator cuff function by the administration of ADSCs.¹⁰³ The fat from the beige depot (brown adipose tissue) and other fat sources have shown to impart paracrine effects to support the remodeling of skeletal tissue.¹⁰⁶ Due to their proximity to rotator cuff tissue, epimuscular fat (EF) from intact and torn rotator cuff tissues were evaluated for their potential role in myogenesis. EF promoted the differentiation of C2C12 myoblasts toward myogenic lineage, indicating another potential cell source for regenerating the rotator cuff.¹⁰⁷

3.3. Other Stem Cells.

In addition to BMSCs and ADSCs, various primary and progenitor cells such as tenocytes, tendon-derived stem cells (TDSCs), synovium-derived stem cells (SDSCs), umbilical cord blood-derived stem cells (UCBSCs), periosteum-derived stem cells (PRSCs), connective tissue progenitor stem cells (CTPSCs), muscle-derived satellite/stem cells (MDSCs), and extra-embryonic cells such as placenta-, and umbilical cord blood-derived stem cells, have been investigated for rotator cuff regeneration. Besides these stem cells, more recently,

induced pluripotent stem cells, which retain several salient features of embryonic stem cells, have been utilized with or without scaffolds for tendon regeneration.^{108,109} For example, human iPSC-derived from neural crest stem cells not only showed tri-lineage differentiation *in vitro*, but also showed significant improvements in macroscopic scores when administered *in vivo* in a rat patellar tendon model.¹⁰⁸ In addition, histologic analyses showed the presence of extended morphology in iPSC-NCSC-treated animals with low vasculature.¹⁰⁸ Furthermore, mechanical analyses revealed significant improvements in failure to load (26.21 ± 3.34 vs 19.20 ± 2.92 N) and modulus values (313.69 ± 92.87 vs 269.35 ± 80.97 MPa) in a iPSC-NCSC-treated group, compared to a non-iPSC-NCSC-treated group.¹⁰⁸

Like other connective tissue stem cells, resident progenitor cells in tendon tissue have been identified, isolated, and characterized for their tri-differentiation potential.^{110,111} The tenocyte-like cells were initially obtained from the long head of the biceps muscle (LHB) and supraspinatus muscle (SSP) by Pauly et al. and were subsequently characterized for their adherence, phenotypic, and biochemical characteristics.¹¹² The MSC-like characteristics of these tenocyte-like cells were reported by Randelli et al. who showed the differentiation of TDSCs into musculoskeletal lineages (osteogenic, adipogenic, and myogenic). Additionally, higher levels of COL1A1, COL3A1, matrix metalloproteinase-2 (MMP-2), transforming growth factor- β 1 (TGF- β 1), connexin43, and low levels of matrix metalloproteinase-1 (MMP-1) expression were observed in TDSCs.¹¹³

In addition to SSPs and LHBs, stem cells have been isolated from the proximal humerus,^{105,114} glenohumeral joint,¹¹⁵ synovium (SDSCs),¹¹⁵ subacromial bursa (SASCs),¹¹⁶ infraspinatus tendons,¹¹⁷ and enthesis tissue (residual tendon stump).^{47,115} A comparative *in vitro* evaluation between the stem cell sources indicated higher viability and expansion capability in SASCs (>passage-10), compared to TDSCs and enthesis-derived MSCs. In addition, SASCs showed higher osteogenic (von Kossa and alkaline phosphatase (ALP) staining), adipogenic (Oil Red O staining), and chondrogenic potential (Toluidine Blue O staining), which were further corroborated by gene expression profiles, suggesting the suitability of SASCs in rotator cuff regeneration.¹¹⁵

In an attempt to enhance the tenogenic differentiation of TDSCs, different approaches such as stimulating the cells with GFs, or by applying pulsed electromagnetic fields have been studied. By combining bFGF (a mitogenic GF), and TDSCs in a rat rotator cuff model, Tokunaga et al. showed 2-fold increases in the ultimate load to failure, and ultimate stress to failure values at 6 and 12 weeks, compared to control animals.¹¹⁸ Similarly, histologic analyses showed increased vascularity, collagen fiber orientation, and total scores at all the time points studied (2, 4, 6, 8, and 12 weeks).¹¹⁸ In addition, the expression level of tendon-specific markers such as Scx and tenomodulin (TNMD) expression levels were significantly up-regulated at all time points.¹¹⁸

In one study, tenocyte-like cells obtained from the tissue biopsies of human patients (young and old age) were combined with bone morphogenetic protein-2 and -7 (BMP-2 and BMP-7), and studied *in vitro* for their effects on proliferation, colony forming capabilities, COL1 synthesis, and relative COL1A1, COL3A1, and decorin gene expression. The studies showed a significant impact of age on cell proliferation, and improvements in tendon-bone

healing by the incorporation of BMP-7, and to a smaller extent BMP-2.^{119,120} Applying pulsed electromagnetic fields have produced therapeutic effects in various musculoskeletal defects/disorders by stimulating self-healing mechanisms. For rotator cuff healing, a study evaluated the effects (proliferation, gene expression profiles, cell viability, cell migration, and cell apoptosis) of TDSCs exposed to pulsed signal therapy (PST). While the PST did not have a significant effects on the proliferation, stemness was maintained at higher levels, evidenced by cell surface markers via FACS and RT-PCR experiments.¹²¹

As discussed before, besides tenocyte-like cells and tendon-related stem cells, various connective tissues such as MDSCs,^{122,123} UCBSCs,¹²⁴ perivascular stem cells (PVSCs),¹²⁵ SASCs,¹²⁶ and PRSCs¹²⁷ have been reported for regenerating rotator cuff tendons. List of representative studies are summarized in Table 2.

4. SCAFFOLDS FOR REGENERATING ROTATOR CUFF TENDONS

In addition to the utilization of optimal cell source, scaffolds can offer another avenue for rotator cuff repair/regeneration. Depending on the structure, morphology, degradation rate, delivery/implantation of the scaffold, their utility can range from providing mechanical stability to the wound bed, to delivering biologics (stem cells or GFs or PRPs), to providing a niche for cells to proliferate. Based on the origin, scaffolds for rotator cuff regeneration can be classified into materials derived from (1) xeno- and allogenic sources, (2) natural origin (collagen, gelatin, etc.), and (3) synthetic (degradable and non-degradable).

4.1. Extracellular Matrix (ECM)-Based Matrices for Rotator Cuff Regeneration.

In clinical settings, most FDA-cleared devices for treating rotator cuff lesions are obtained from xenogenic or allogenic origin (Table 1). The xenografts are obtained mostly from porcine (SIS) and bovine (dermis) species (Table 1). The xenografts are obtained by subjecting the tissues through patented and proprietary techniques to remove cellular and other unwanted components, and thereby eliminating/mitigating the immune response after implantation. Even though most cellular components are removed, patches or grafts retain most of the ECM components—primarily collagen (type-I), elastins, PGs, and cytokines/GFs—thereby providing biochemical cues to facilitate tissue in-growth. Although, the presence of type-I collagen in xenografts mimic the native environment for t cells to adhere and proliferate, they are often subjected to enzymatic degradation. With very few exceptions, currently, most xeno-, allo-, and synthetic grafts show poor mechanical properties (primarily low stiffness and modulus). To minimize enzymatic degradation and improve the mechanical properties, xeno- and allografts are sometimes cross-linked (to varying degrees) to increase the residence time at the graft site (Table 1). Despite significant developments in graft fabrications, rejection rates due to immune reactions, and graft failure rates (>30%), necessitate the development of novel scaffolds that can provide long-term mechanical augmentation for long periods of time. In this regard, several acellular grafts derived from human and various animal species with lower immunogenicity are being evaluated for their potential to regenerate rotator cuff tendons^{130–139} (summarized in Table 3).

4.2. Biomaterials of Biologic and Natural Origin for Rotator Cuff Regeneration.

Numerous biomaterials of biologic and natural origin such as type-I collagen, gelatin, PRP, fibrin, hyaluronic acid, and self-assembled peptides have been evaluated for tissue regeneration, including rotator cuff regeneration.¹⁴⁰ Type-I collagen-based hydrogels, for example, upon implantation in a rat model have demonstrated the production of mechanically superior tissue, when compared to acellular matrix and sutures in some studies. Interestingly, improvements in the biomechanics (maximum load bearing capability) were more significant, when allogenic MSCs were used in conjunction with the collagen scaffold.¹⁴¹ The feasibility of complete absorption of the implanted collagen hydrogel with no foreign body reaction was reported in an ovine model. Good integration of the neo-tissue with the cortical bone in 6 months was also observed. At 12 months follow-up, a fibrocartilaginous transition zone was seen with a significant increase in the thickness of the infraspinatus tendon found.¹⁴²

Despite encouraging results, fast degradation rates and low load bearing capability remain major challenges associated with collagen-based scaffold systems has led to the development of other types of scaffolds.^{143,144} Fabricating scaffolds using conventional textile technology such as weaving, in combination with chemical cross-linking has been studied. A recent study investigated the load bearing capabilities of cross-linked woven collagen scaffolds in a rabbit model. The study demonstrated via load–displacement curves, scaffold-induced repair to be comparable to direct repair, but inferior to the intact native shoulder tendon.¹⁴⁵

Gelatin, a denatured form of collagen, is another biologic scaffold^{146,147} reported for rotator cuff regeneration. Like collagen hydrogels,^{148–151} gelatin hydrogels, have been reported for the sustained release of BMP-7, PDGF-BB, and bFGF for bone–tendon healing.^{118,152,153} When release rates of BMP-7 were investigated from gelatin hydrogel sheets (GHSs), the residual content of BMP-7 in the gel was higher at all time points (1, 3, 7, 14, and 21 days) compared to the control group. In addition, the gradual release of BMP-7 from the GHS scaffolds led to a favorable orientation of the formed collagen fibers. Furthermore, subsequent Safranin-O staining of the tendon–bone insertion site demonstrated larger stained areas in the GHS group, compared to the other experimental groups. Moreover, higher tendon–bone maturing scores (22.3 ± 2.4 for GHS/BMP-7 vs 18.3 ± 3.4 for BMP-7) and ultimate force-failure values (21.1 ± 3.1 N for GHS/BMP-7 vs 17.5 ± 2.0 for BMP-7) demonstrated differences obtained via the use of the GHS scaffold in rotator cuff regeneration.¹⁵²

Similar to BMP-7, bFGF incorporation in gelatin hydrogels has been shown to provide significant improvements in terms of biomechanics, collagen fiber orientation and total scores (histology), and gene/transcription expression levels (immunostaining and RT-PCR) after 8 and 12 weeks of implantation in a rat rotator cuff healing model. Upon 12 weeks of implantation, orientation of collagen fibers in the regenerated tissue increased from 70.8 ± 6.4 in GHS to 81.9 ± 4.5 in GHS containing bFGF. Likewise, up-regulation in the gene expression levels of Scx and tenomodulin by RT-PCR and immunostaining, indicated the potential of GHS containing bFGF for rotator cuff regeneration.¹¹⁸

Other GFs that have been incorporated in GHS include PDGF-BB, an isoform of PDGF known for its mitotic and chemotactic effects on the cells.¹⁵⁴ When the gelatin hydrogels containing PDGF-BB were evaluated in terms of histology, cell proliferation, and biochemical outcomes at 6 and 12 weeks, the composite scaffold outperformed control groups (suture alone or hydrogels with no PDGF-BB) at all time points. The orientation of collagen fibers, for instance, was significantly higher in the GHS/PDGF-BB group ($79.4 \pm 5.6\%$) at 12 weeks, when compared to both suture ($64.6 \pm 4.2\%$) and suture/GHS ($63.0 \pm 5.3\%$) groups. Similarly, the total histologic scores were higher in the GHS/PDGF-BB group (10.3 ± 0.8) compared to the suture alone (8.5 ± 0.5) and the GHS without PDGF-BB (8.8 ± 0.8) groups. These results were further corroborated by an increase in the ultimate load to stress (7.99 ± 2.13 vs 3.28 ± 1.27 vs 3.31 ± 0.80 N/mm²), stiffness (11.05 ± 2.37 vs 5.86 ± 1.75 vs 4.74 ± 1.31 N/mm), and ultimate load to failure values (28.28 ± 6.28 vs 11.85 ± 2.89 vs 10.44 ± 1.98 N) in the GHS/PDGF-BB group, illustrating the advantages of combining GHS with PDGF-BB for rotator cuff regeneration.¹⁵³

In addition to these, fibrin-based scaffolds have also been evaluated for their potential in rotator cuff regeneration. One of the key advantages of fibrin scaffolds is the possibility of fabricating scaffolds during the patient visit, and its easier application into the repair site.¹³⁸ Because of these reasons, fibrin-based scaffolds have been evaluated in various animal model and human pilot studies for rotator cuff tissue regeneration. However, a comparative evaluation demonstrated poor differentiation and proliferation capabilities of BMSCs in fibrin matrix-based scaffolds compared to ECM-based scaffolds of biological origin (decellularized dermal patch, collagen, and rotator cuff tendon scaffolds) in one study. Quantitative PCR (qPCR) demonstrated significant up-regulation of ALP and osteocalcin (osteogenic lineage); COL2A1 and aggrecan (cartilage); PPAR γ and FABP4 (adipogenic lineage); decorin, TSN-c, and COL3A1 expressions (tenogenic lineage) in cells cultured on rotator cuff tendon and collagen scaffolds, respectively, compared to fibrin matrix-based scaffolds.¹³¹ Similar results were noted in a study by Chong et al., which showed identical collagen fiber arrangement and biomechanical behavior at 6 and 12 weeks in BMSC-seeded fibrin scaffolds, despite showing better fiber arrangement and biomechanical behavior at earlier time points (3 weeks), compared to fibrin-alone scaffolds.¹⁵⁵

Several other biomaterials have also been investigated for the development of scaffolds for rotator cuff regeneration. For example, Funakoshi et al. developed and evaluated a chitosan-based hyaluronan fiber scaffold, with and without seeded fibroblasts, for rotator cuff regeneration.¹⁵⁶ The fibroblast-seeded scaffolds showed superiority in terms of type-I collagen secretion and improvements in mechanical behavior at 12 weeks in tensile strength (9.2 ± 0.8 vs 7.1 ± 0.8 MPa at 12 weeks) and tangent modulus (89.0 ± 7.4 vs 60.2 ± 7.4 MPa at 12 weeks), compared to scaffolds without fibroblasts.¹⁵⁶ Similarly, the same research group evaluated chitin-fabric scaffolds for rotator cuff regeneration.¹⁵⁷ Without cell seeding, the authors demonstrated significant increases in cell numbers via histologic analysis.¹⁵⁷ Likewise, improvements in the failure load (135.4 ± 28.2 N vs 12.9 ± 7.1 N for control) and stiffness (47.1 ± 13.6 vs 6.2 ± 2.8 N) were also observed in the implanted scaffold.¹⁵⁷

Similarly, hyaluronic acid (HA)-based hydrogels have shown both adhesion formation and tendon healing *in vivo*.¹⁵⁸ Rapid degradation of the HA can be overcome by cross-linking

HA resulting in the residence of HA for several weeks *in vivo*.¹⁵⁸ An auto-cross-linked HA for regenerating flexor tendons in a Lapine model demonstrated faster healing and mechanical improvements (breaking strength) at 2 weeks (19.0 ± 2.5 N in HA gel-treated vs 15.4 ± 0.9 N in saline-treated animals) and at 12 weeks (60.0 ± 2.1 N in HA gel-treated vs 46.5 ± 4.1 N in saline-treated animals), illustrating the potential of these hydrogels in rotator cuff regeneration.¹⁵⁸

4.3. Biomaterials of Synthetic Origin for Rotator Cuff Regeneration.

Rapid resorption and variability are some of the challenges in the use of hydrogels of natural origin. Synthetic hydrogels such as poly(ethylene glycol) diacrylates (PEGDA), have therefore been evaluated for their potential in regenerating rotator cuff tendons.¹⁵⁹ A PEGDA-based injectable hydrogel containing periosteal-derived progenitor cells (PPCs) and BMP-2 was administered for healing tendon–bone junction in an infraspinatus defect rat model. Histologic analysis showed progressive fibrocartilage formation resulting in bone formation adjacent to the native bone at 8 weeks.¹⁵⁹ Likewise, immunohistochemical analyses showed the presence of aggrecan and COL2A1 indicating the presence of a fibrocartilage layer.¹⁵⁹ Finally, biomechanical testing revealed superior pull out load values in PEGDA scaffolds containing BMP-2 and PPCs at 4- and 8-week time points, progressing rapidly toward that of native non-operated pull out values, suggesting the suitability of PEGDA+GFs for regenerating the enthesis.¹⁵⁹

Synthetic scaffolds (non-hydrogels) can be fabricated with desired morphologies, shapes, and sizes, permitting surgeons to customize the final product prior to the surgery.¹⁶⁰ Other advantages of using synthetic scaffolds include low or no risk of disease transmission, precise degradation rates and potential high biocompatibility. On the other hand, lack of tissue in-growth due to the lack of surface epitopes can be of concern with these scaffolds.^{160,161} Both degradable and non-degradable scaffolds (summarized in Table 3) of synthetic origin have been evaluated for rotator cuff regeneration.

Poly tetra fluoro ethylene (PTFE), polyethylene terephthalate (PET), polycarbonate polyurethane are some of the non-degradable polymers that have been evaluated for promoting rotator cuff regeneration.^{161,162} For example, a report evaluating the augmentation of suture strands with an expandable PTFE (ePTFE) patch in full thickness ovine shoulders (cadaver) showed higher footprint contact pressures and higher ultimate load to failure (~50% higher compared to non-augmented controls) on the bursal side.¹⁶³ Prior to this study, studies have shown almost no biological reaction to ePTFE even after a prolonged period of time (up to 1 year) *in vivo* demonstrating its biocompatibility.¹⁶⁴ The extension of neo-tissue fibers between the defect and ePTFE fibers was observed in a canine study demonstrating the safety as well as the efficacy of the implant for rotator cuff regeneration. Finally, a long-term clinical evaluation (10 years) of ePTFE in 10 patients with inoperable rotator cuff tears demonstrated less severity in pain with overall improvements in shoulder function (external rotation and range of motion (ROM), suggesting the safety and suitability of these scaffolds for use in particular for irreparable rotator cuff lesions.¹⁶²

Similar to ePTFE, poly carbonate polyurethane scaffold patches have resulted in clinical improvements in subjective scores (VAS, SF-12, and UCLA scores) as well as MRI and

ultrasound scores in clinical trials.¹⁶⁵ MRI, for instance showed healing in upward of 90% of patients at 12 months post-operatively with no adverse events in the rotator cuff tissue.¹⁶⁵ Likewise, the range of motion increased substantially at 12 months compared to both pre-operative and 6 months post-operative levels.¹⁶⁵ Although these studies demonstrated the safety and efficacy of non-degradable scaffolds for rotator cuff regeneration, poor tissue in-growth, late stage inflammatory response, non-degradability, and lack of long-term follow-up studies necessitated the investigation of degradable synthetic scaffolds for rotator cuff regeneration.

Fibrous structures based on aliphatic polyesters such as PLA,^{166–168} poly(glycolic acid) (PGA),¹⁶⁹ poly(lactide-co-glycolide) (PLGA),¹⁷⁰ poly(ϵ -caprolactone) (PCL),¹⁷¹ P4HB,¹⁷² poly(lactide-co-caprolactone) (PLCL) and their copolymers remain the most widely studied material type for tissue regeneration, including rotator cuff regeneration. Other biomaterials that have been evaluated for regenerating rotator cuff include silk¹⁷³ and poly(ester urethane urea).¹⁶⁵ Conventional textile-based systems such as braided, knitted, and woven structures, non-woven matrices (nanofibers), and films and sheets are some of the common scaffold types employed in rotator cuff regeneration. Unlike using plain fibers as scaffolds, processing fibers into braided, knitted, or woven structures afford the opportunity to mimic the specific geometry and architecture of the native tissue (tendon–bone insertion, fibrocartilage, and tendon).

Similar to our research group's work on advanced scaffolds based on braided PLA fibers for ligament regeneration,^{168,174–180} PLA and PLA/PGA fibers have been braided and sutured with fascia lata to form a composite structure for the purpose of reinforcing rotator cuff tissue. Subsequent mechanical evaluation of explants from a rat abdominal wall defect model showed superiority in terms of maximum load, stiffness, and elongation at 50 and 180 N.¹⁸¹ As expected, braided PLA/PGA fibers showed higher load, stiffness, and lower elongation under conventional and physiological conditions at shorter time points (0 and 4 weeks).¹⁸¹ Due to the possible degradation of PGA at longer time points, the braided PLA fibers showed higher load, stiffness, and lower elongation at later time points (12 weeks).¹⁸¹

Based on the positive outcomes in rat model, a subsequent study evaluated the braided PLA/fascia lata composite in a canine cadaver shoulder model (12 weeks) for tendon retraction, cross-sectional area, stiffness, ultimate load, and foreign body reactions.¹⁸² While stiffness in non-augmented and augmented groups was similar at 0 (211 ± 22 vs 232 ± 43 N/mm) and 12 weeks (265 ± 49 vs 248 ± 43 N/mm), ultimate load bearing was higher in the augmented group at the earlier time points 0 weeks (966 ± 160 vs 670 ± 112 N).¹⁸² However, at 12 weeks (1228 ± 115 vs 1037 ± 189), no significance difference in the failure load was observed.¹⁸² More importantly, augmented scaffolds showed no foreign body reaction (FBR) at both time points, indicating that improvements in early stage mechanical properties with the lack of FBR, might improve the potential clinical outcomes of these composite structures for rotator cuff regeneration.¹⁸²

To validate the hypothesis that composite structures provide initial mechanical augmentation, advanced biomechanical testing was carried out in a human cadaveric model for cyclic gap formation and failure properties of the composite grafts.¹⁸³ The results

showed a 32% decrease in the stroke elongation (4.5 ± 1.0 for non-augmented vs 3.0 ± 0.7 mm for augmented scaffolds) at the end of the first cycle.¹⁸³ Similarly, gap formation in the augmented group was consistently lower compared to the non-augmented group at the end of one cycle (1.8 ± 0.6 vs 3.6 ± 1.0 mm), 10 (2.3 ± 0.7 vs 4.3 ± 1.1 mm), 100 cycles (3.4 ± 1.0 vs 5.8 ± 1.1 mm), and 1000 cycles (4.7 ± 0.14 vs 7.3 ± 1.3 mm), indicating the possibility of lowered tendon retraction and rotator cuff re-tear after implantation.¹⁸³

Like braided fibers, knitted and woven microfibers have also been evaluated for tissue regeneration. Knitted scaffolds typically have higher porosities, and subsequently demonstrate lower mechanical properties and are incapable of transferring load in the way typically seen with braided and woven fibers.^{184,185} In contrast, lower porosities and superior mechanical properties are seen with woven scaffolds, further expanding the repertoire of scaffold choices in tissue regeneration.¹⁸⁵

In addition, scaffolds based on textile structures have been used in conjunction with stem cells for rotator cuff regeneration.^{109,186} For example, Vuornos et al. evaluated two different scaffolds (braided PLA and PLCL foams) seeded with human ADSCs for their potential in regenerating rotator cuff tendons.¹⁸⁶ After 14 days of cell seeding, cell viability experiments (live/dead assay) showed the formation of uniform cell layers on braided PLA scaffolds, and small cell aggregation in PLCL scaffolds.¹⁸⁶ Likewise, cell numbers and tenogenic gene expression levels of COLA1, TNMD, and Scx were higher in PLA scaffolds than in PLCL scaffolds at both 7 and 14 days, demonstrating the suitability of braided PLA structures containing ADSCs for rotator cuff regeneration.¹⁸⁶

As a primary role of these scaffolds is to provide mechanical augmentation immediately after implantation, woven constructs of synthetic degradable polymers have been evaluated for mechanical augmentation.¹⁸⁷ For example, Inui et al. studied two different woven PLA-based scaffolds (smooth plain woven and double layered PLA) for rotator cuff regeneration.¹⁸⁷ Even though the study investigated the scaffolds *in vivo* (rabbit model) for only up to 6 weeks (time points of 3 and 6 weeks), at both time points, cell attachment and migration was higher in double-layered PLA, compared to smooth plain scaffolds.¹⁸⁷ Likewise, a 4-fold difference in DNA amounts was seen in double-layered scaffolds compared to smooth plain scaffolds.¹⁸⁷ Finally, mechanical evaluation (ultimate failure load and energy absorbed) showed differences between the two scaffold groups demonstrating the suitability of the double layered woven PLA scaffolds for rotator cuff regeneration via mechanical augmentation.¹⁸⁷

Similar to the study by McCarron et al.¹⁸³ using braided PLA fibers, mechanical properties of augmented scaffolds (woven PLA fibers) in three anatomical regions (anterior supraspinatus, posterior supraspinatus, and superior supraspinatus) of the rotator cuff were compared against non-augmented groups in a human cadaveric model.¹⁸⁸ Similar to the braided constructs described earlier, woven constructs showed superior mechanical properties in all three anatomical regions, in terms of yield load ($P \ll 0.04$) and ultimate load ($P \ll 0.01$), although initial stiffness remained similar in augmented and non-augmented groups across all the three anatomical regions.¹⁸⁸ In spite of these studies demonstrating the

potential of woven constructs in regenerating rotator cuff, the results should be taken with caution, as the studies either dealt with a human cadaveric model or small animal model.

In an *in vivo* study reported by MacGillivray et al., a biomechanical evaluation (ultimate load to failure) carried out on explants from a goat model showed no statistical significance with the use of woven PLA constructs compared to non-augmented constructs at all time-points (3, 6, 12, and 24 weeks).¹⁸⁹ These results were corroborated by histologic analyses which showed organized, fibrous reparative scar tissue at the repair site by 12 weeks, and the formation of highly organized, cellular fibrous tissue by 24 weeks in both the groups, questioning the efficacy of the woven constructs for rotator cuff regeneration.¹⁸⁹

Nanofibrous scaffolds are other major textile-based structures that are being studied in recent years for rotator cuff regeneration.^{190,191} As the collagen fibers are comprised of a nano-micro-structured hierarchical arrangement in the native rotator cuff ECM, nanofiber matrices afford the possibility of recapitulating the architecture of native ECM.^{190,192–200} Nanofibers can be fabricated using variety of techniques, including: template synthesis, electrospinning, melt electrospinning, phase separation, and self-assembly.¹⁶⁰ Among these techniques, versatility and low setup cost make electrospinning the most common technique for fabricating fibers with sub-micrometer diameters.^{201,202} The nanofibers are typically obtained in the form of non-woven mats by applying high voltage to a polymeric solution exiting the needle.^{203,204} As the solution droplets exit the needle, it undergoes elongation and whipping resulting in the drastic reduction of fiber diameters from several micrometers to sub-micrometers (typically less than 500 nm).^{205–209} An added advantage with the use of electrospun nanofibers is their versatility in terms of anisotropic mechanical properties, fiber morphology, encapsulate and deliver bioactive molecules (antimicrobial agents, drugs, nanoparticles), all of these can be achieved by modulating the electrospinning setup or parameters.^{190,203,210–212} For tissue regeneration, the advantage of using nanofibers include the mimicry of the native ECM structure with high surface area to volume ratio, high porosity (%), higher cell attachment along the fiber direction and efficient nutrient transportation through the porous structures.¹⁹⁰ Owing to these advantages, electrospun nanofibers are widely used to study regeneration of various tissues,²¹³ including skin,^{214–216} cartilage,²¹⁷ bone,^{218–221} meniscus,²²² tendon, and rotator cuff tissues.^{198,223,224} Laurencin and his team were the first to explore nanofiber for engineered tissue use.¹⁹² Their work has been comprehensive and has examined nanofiber structure development from fundamental research^{215,225,226} to clinical applications.^{216,227–231}

Aligned nanofibers impart nano-topographical and morphological cues, which have often demonstrated improved cell attachment, alignment, gene expression profiles, and matrix synthesis of tendon fibroblasts.^{232,233} Similarly aligned electrospun PLA nanofibers compared to randomly oriented PLA nanofibers have shown not only higher mechanical properties (stiffness, 3.48 ± 1.09 vs 0.07 ± 0.04 N/mm; force to failure, 1.88 ± 0.05 vs 0.17 ± 0.06 N; modulus, 22.76 ± 5.63 vs 0.63 ± 0.56 MPa), but also superiority in terms of tenogenic differentiation potential of human TDSCs.²³⁴ Furthermore, RT-PCR experiments showed significant down-regulation of bone matrix expression such as COL1A1, COL3A1, ALP, osteocalcin (OCN), with concomitant up-regulation of muscle/tendon-specific genes such as Eya2, Scx, COL14, and elastin in cells cultured on aligned nanofibers.²³⁴ Finally, *in*

vivo experiments demonstrated a greater presence of cells with spindle-shaped morphology, and highly organized collagen deposition at 1 week in aligned nanofibers, with randomly aligned nanofibers showing the presence of round shaped cells and highly disorganized ECM.²³⁴

In addition to fiber alignment, fiber diameter of the scaffolds is another factor that may play a key role in inducing tenogenic differentiation.²³⁵ A comparative study of electrospun aligned PLGA non-woven mats with varying fiber diameters (320 ± 100 , 680 ± 180 , and 1800 ± 160 nm) revealed an up-regulation of key tenogenic markers such as COL1A1, COL3A1, COL5A1, and TNMD, in large fiber diameter scaffolds seeded with rotator cuff fibroblasts at all time points (3, 7, and 14 days).²³⁵ In addition, the study also demonstrated large aspect ratios (ratio of length to width) with low cell numbers, when seeded on non-woven mats with large fiber diameters, indicating the key role of fiber size in inducing tenogenic differentiation.²³⁵ Even though the specific pathway for such fiber alignment and fiber-size-induced differentiation is not well known, they are attributed to the parallel aligned collagen fibers in the native tissue, and subsequent possible mechanotransduction effects of those fibers on the resident stem cells via cell–matrix interactions.²³⁴

One way to mimic the native environment more closely is by implanting stem cell-seeded aligned nanofibers for rotator cuff repair, as demonstrated by a recent study from the Laurencin group.²³⁶ Studies in our lab optimized the properties of a PCL nanofiber scaffold (fiber diameter, polyphosphazene coating) and subsequently studied the cell–matrix interactions and differentiation potential of human MSCs on these nanofibers.^{237,238} Polyphosphazene coating of the fibers using solutions with concentrations up to 2% improved the hydrophilicity and surface interactions with cells, leading to greater penetration (167 vs $67 \mu\text{m}$ for unmodified scaffolds) of MSCs and greater DNA synthesis in the scaffolds.²³⁸ However, further increase resulted in decreased mechanical properties and suture strength, indicating the 2% concentration to be the optimal solution concentration.²³⁷ Likewise, PCL fiber diameters were demonstrated to be a key determining factor with respect to mechanical properties, cell infiltration, and DNA synthesis.²³⁷ In addition, tendon-specific markers (TNMD and Scx) were up-regulated in phosphazene-coated PCL nanofibers, compared to PCL films and nanofibers, demonstrating the suitability of phosphazene coated-PCL scaffolds in tendon tissue regeneration.²³⁸

Based on these studies, our group recently evaluated polyphosphazene poly((ethyl alanato)₁(*p*-methyl phenoxy)₁) phosphazene (PNEA-mPh) coated PCL nanofibers in a rat supraspinatus rotator cuff model.²³⁶ The surface functionalized matrices were seeded with BMSCs and cultured for 2 days, upon which the cell-laden scaffolds were implanted in a rat rotator cuff integrative augmentation model.²³⁶ Masson trichrome analyses at 12 weeks showed gradual transition of aligned parallel collagenous tissue to the bone with a mid-substance in BMSC-laden constructs (Figure 3A IV) similar to the native tissue (Figure 3A–I). Animals that underwent suture (Figure 3A–II) and mechanical augmentation (no cells) (Figure 3 A-III), however, showed abrupt transition.²³⁶ Additionally, hematoxylin and eosin (H&E) staining of native tissue (Figure 3B–I) and cell-laden constructs (Figure 3B–IV) showed arrangements of collagen fibers in parallel with low tenocyte cell density with extended morphology at 12 weeks post transplantation.²³⁶ Animals treated with primary

sutures (Figure 3B II) and mechanical augmentation (Figure 3B III), on the other hand showed large numbers of highly disorganized rounded cells, demonstrating a less than optimal healing pattern in these groups.²³⁶ Similar to morphological analyses, biochemical analyses indicated higher COL1:COL3 ratios (Figure 3C) and birefringence values (Figure 3D) demonstrated an excellent healing response closely resembling that of native intact rotator cuff tissue.²³⁶ In accordance with biochemical and morphological analyses, biomechanical analyses revealed significant differences between the experimental groups and the native tendon tissue.²³⁶ Although the cross-sectional area of the scaffold/MSG group was higher than native tendons, the areas observed at 6 and 12 weeks were substantially lower than other experimental groups (repair or mechanical augmentation).²³⁶ A similar trend was also seen in ultimate stress values with the scaffold/MSG group outperforming both the experimental groups,²³⁶ demonstrating the significant potential of these scaffolds in rotator cuff tendon regeneration.

The cell–matrix interactions of the scaffolds can also be improved by coating or grafting or co-electrospinning cell-adhesive peptides containing proteins such as collagen, gelatin, and FN.^{239–243} A rat *in vivo* (rotator cuff model) study demonstrated not only improved cellular proliferation, but also cartilage regeneration at the insertion site in gelatin-grafted PLA nanofibers, compared to neat PLA nanofibers.²³⁹ In addition, picrosirius red staining and birefringence analyses showed total areas of metachromasia and collagen orientation, respectively, to be higher in gelatin-grafted PLA, indicating the potential of gelatin grafted nanofibers for regenerating the tendon–bone insertion site.²³⁹ Likewise, a recent study evaluated co-electrospun PCL/collagen and PLGA/collagen nanofibers for rotator cuff regeneration.²⁴⁰ The presence of collagen in the nanofibers improved the cell viability as observed by live–dead and CCK-8 assays.²⁴⁰ In addition, further *in vitro* analyses showed larger amounts of collagen secretion in collagen containing scaffolds, and subsequent *in vivo* implantation showed compatibility and superior strength of the reorganized tissue in collagen containing scaffolds via histologic analyses, thereby suggesting that incorporation of ECM protein-containing nanofibers would improve the possibility of rotator cuff regeneration.²⁴⁰

Several techniques have been developed to improve the low porosity, low mechanical properties and lack of microstructural hierarchy sometimes observed in nanofiber scaffolds. One such technique employed a sacrificial polymer poly(ethylene oxide) (PEO), that could be removed after spinning to create a favorable porosity range, to facilitate cellular proliferation through the full thickness of the scaffolds resulting in tissue in-growth.^{257,258} Interestingly, despite higher PEO content (40%), the histological characteristics and mechanical properties of leached PCL scaffolds at 4 weeks of implantation (load: 10.8 ± 4.4 vs 9.01 ± 3.38 N for PCL; max stress: 1.28 ± 0.57 vs 1.03 ± 0.40 MPa; stiffness: 20.4 ± 8.6 vs 17.3 ± 8.3 N/mm; modulus: 11.4 ± 6.8 vs 10.3 ± 8.0 MPa) were comparable to that of unleached PCL/PEO scaffolds.¹⁶⁹

Other key methods employed to improve porosity and or microscopic architecture of nanofibers has been by sequential collection of individual layers;^{241,259,260} stacking composite of nano- and microfibers;²⁶¹ embedding nanofiber matrix in a 3-D matrix such as hydrogel²⁶² or microspheres;^{263–265} and, converting nanofibers into a 3-D scaffolds

by braiding,²⁶⁶ weaving,²⁶⁷ or other textile process,^{268,269} with each technique providing additional benefits toward rotator cuff regeneration. For example, stacking nanofibrous layers affords the potential to incorporate bioactive molecules, making it suitable for regenerating tendon–bone with a cartilaginous midsubstance in rotator cuff repairs.²⁷⁰ Likewise, by braiding the nanofibers, native tissue mimicry may be achieved to a higher degree due to the presence of nano- and microscopic architectures in the scaffold.^{109,266} Similarly, by embedding the nanofibrous matrix in a hydrogel, sustained delivery of cells and other biologics (growth factors, for example) may be possible. These studies thus demonstrated a wide array of possibilities available with nanofibers that could provide avenues for successful rotator cuff regeneration.

5. GROWTH FACTORS AND SMALL MOLECULES IN ROTATOR CUFF REGENERATION

Tissue regeneration requires careful orchestration of events in a sequential fashion involving factors such as resident cells, stem cells, GFs, and other agents, leading to the formation of mature tissue.¹⁹⁰ Upon injury and homeostasis, inflammatory cells such as macrophages and neutrophils sequentially release GFs to provide stimulant for tissue regeneration. The GFs are signaling molecules that actively partake in cell proliferation, differentiation, ECM synthesis, and tissue remodeling.²⁷¹ To date, various GFs such as GDF-5, TGF- β 1, IGF-1, FGF-2 (also called FGF- β or β FGF), PDGF-BB, GDF-6, GDF-7, and PRPs (cocktail of GFs) have been implicated for their role in tendon/rotator cuff regeneration.⁸³ Most commonly observed challenge with the use of GFs are their short half-life, large molecular size, and tendency to undergo degradation. Secondary challenges include, optimal timing and dosage of the GFs, and identifying combination and sequence of GFs needed for successful tissue regeneration.²⁷² Coating with scaffolds (or sutures), delivering through nanofiber scaffold (via core–sheath structure), encapsulating in microspheres/nanospheres, grafting/immobilization (for example, heparin or polydopamine coating) in scaffolds, and gene delivery are some of the common mechanisms employed for local delivery of GFs.

A. Growth and Differentiation Factors.—Growth differentiation factors (GDF-5) belong to the superfamily of transforming growth factors and are most implicated for their role in tendon regeneration.²⁷³ Among various GDFs, GDF-5 is the well-known GF for tendon regeneration, and deficiency of GDF-5 have shown delayed healing response in rodents.²⁷³ After identifying optimal GDF-5 dosage by observing improvements in the proliferation and tenogenic differentiation potential of tendon fibroblasts via MTT assay and gene expression analyses, respectively,^{274,275} experiments were subsequently carried out in 3-D scaffolds seeded with ADSCs.²⁷⁶ Like tendon fibroblasts, upon exposure to GDF-5, a dose-dependent improvement in ADSC proliferation and migration, with gene expression analyses showing up-regulation of COL1A1 and Scx were observed in 3-D nanofiber scaffold.²⁷⁶

As coating GFs offers close relevance to clinical setting, GDF-5 coated Vicryl 4-0 sutures were evaluated via *in vivo* and *in vitro* experiments for improving biomechanics and *de novo* cartilage formation, and cell migration of tendon fibroblasts, respectively.²⁷⁷ Despite

no obvious improvements in the histologic scores across the groups, *de novo* cartilage formation and improvements in the biomechanical properties (mechanical strength and ultimate load, but not stiffness) was observed at 3 weeks of implantation.^{277,278} To further understand the underlying mechanism, proliferation and migration assays were carried out *in vitro* on GDF-5 coated sutures, which showed dose-dependent effects of GDF-5 on migration and proliferation of tendon fibroblasts.²⁷⁸

GDF-5 has also been delivered by a col-spo to the lateral aspect of the tendon site over the knots, subsequently tied to hold the col-spo. *In vivo* rat study (supraspinatus rotator cuff model) demonstrated significant improvements in the mechanical strength (~80% of the control rotator cuff tendons at 6 weeks), with histologic analyses further demonstrating greater cellular organization and synthesis of aligned collagen fibers along the tendon axis in col-spo-mediated GDF-5 delivered animals, indicating both the delivery method and GF dosage to play a key role in rotator cuff regeneration.²⁷⁹

Like GDF-5, BMP-12 (GDF-7) and PDGF-BB have been delivered via a col-spo, and have shown promise *in vivo* (ovine model) for tendon–bone healing in rotator cuff regeneration.¹⁵¹ After induction of rotator cuff tear in ovine model, GDF-7 was delivered from sponges of collagen type-I, collagen type-III, hyaluronic paste, and sponge.¹⁵¹ Biochemical analyses of the retrieved explants showed higher DNA contents ($3.28 \pm 0.91 \mu\text{g}$) in GDF-7/hyaluronan paste group; however, no such significance was seen in max load or stiffness values, compared to GDF-7 delivered from hyaluronan sponge. Although the study demonstrated superiority of GDF-7/collagen type-I sponge on rotator cuff regeneration, radiographic analyses showed significant incidence of ectopic bone formation in both type-I (11/16) and type-I/III sponges (7/12), compared to GDF-7 delivered from hyaluronan paste (2/7) and sponge (1/8), suggesting hyaluronan sponges to be a suitable option for delivering BMP-12.¹⁵¹

5.2. Platelet-Derived Growth Factors.

PDGF is a potent mitotic,²⁸⁰ and chemotactic agent for various cells including fibroblasts,²⁸¹ endothelial cells,²⁸² and MSCs.²⁸³ In addition, PDGF-BB is also an angiogenic agent²⁸⁴ implicated in the later stages of the inflammatory stage and the early stage of healing phase, evidenced by higher PDGF-BB concentrations during this time period (7–14 days after injury).²⁷¹ An added advantage with the use of PDGF-BB includes, FDA approval for two devices for wound healing (Regranex Gel) and periodontal regeneration (GEM 21S), respectively, and consistency, and reliability typically seen with PDGF-BB over PRPs.²⁸⁵

Like GDF-5 coated sutures, PDGF-BB has been delivered via dip-coated sutures²⁸⁶ and col-spo,²⁸⁷ and were subsequently evaluated in an ovine model. Prior to implantation in an ovine model, No. 2 FiberWire suture was coated with a PDGF-BB solution by dip coating process,²⁸⁶ and with gelatin/BMP-12 solution in the second study,²⁸⁷ and subsequently evaluated for their rotator cuff regeneration potential. Biomechanical evaluations after 6 weeks of repair did not show significant improvements in load bearing capacity, energy absorbed, ultimate stress, toughness, and elongation.²⁸⁶ However, a marginal increase was seen in the modulus (35.8 ± 14.3 vs 13.1 ± 5.5) MPa for suture only group), with significant

decreases in the cross-sectional area ($561.9 \pm 102.5 \text{ mm}^2$ vs $724.5 \pm 118.6 \text{ mm}^2$ for suture only group; $P = 0.01$), demonstrating a need for a delivery vehicle for PDGF-BB.²⁸⁶

To augment the PDGF-BB delivery, a collagen matrix was used and subsequently implanted in an ovine model.²⁸⁷ Biomechanical evaluations of the PDGF-BB/type-I collagen matrix demonstrated dose-dependent improvements in load to failure values (1490.5 ± 224.5 and $1486.6 \pm 229.0 \text{ N}$ for 75 and 150 μg PDGF-BB delivered in collagen matrix, respectively), stiffness (134.4 ± 15.5 and 147.5 ± 13.7 for 75 and 150 μg PDGF-BB, respectively), and elongation (15.2 ± 1.0 and 15.4 ± 2.4 for 75 and 150 μg PDGF-BB, respectively), that were statistically significantly greater than the controls (suture only or collagen matrix without PDGF-BB).²⁸⁷ However, further increases in the PDGF-BB dosage (at 500 μg dosage: $677.8 \pm 105.9 \text{ N}$, $104.3 \pm 21.4 \text{ N/mm}$, 11.5 ± 1.3 for load, stiffness, and elongation values, respectively) led to inferior biomechanical characteristics,²⁸⁷ demonstrating the requirement of optimal GF dosage for efficient rotator cuff regeneration. These observations were further corroborated by the histologic scores that showed lower inflammatory cells, higher degrees of fiber orientation and density, along with the generation of Sharpey's fibers in the regenerated tissue.²⁸⁷

A major drawback of collagen matrices are their low mechanical augmentation capabilities, despite their biological relevance. One way to obviate the drawbacks is to use synthetic materials with high porosities for delivering PDGF-BB.²⁸⁸ For example, Uggen et al. studied tendon fibroblasts transfected with PDGF-BB and IGF-1 genes seeded on porous PGA constructs for rotator cuff regeneration in a rat model.²⁸⁸ Unlike what was seen with collagen constructs, histologic evaluations showed significant improvements in tendon quality crimp pattern restoration and collagen bundle alignment, leading to near-complete regeneration as seen in normal rotator cuff tissue.²⁸⁸ These cumulative results demonstrated the possible therapeutic role of PDGF-BB/collagen matrix in rotator cuff regeneration.

Immobilization of GFs overcomes several limitations such as short half-life and degradation,¹⁹⁰ typically associated with their use in tissue regeneration. By taking advantage of the innate binding capability of PDGF-BB and BMP-2 in heparin domains via ionic interaction, Min et al. fabricated a highly porous PCL/Pluronic F127 membrane with heparin immobilized PDGF-BB (right to membrane) and BMP-2 (left to membrane) domains for tendon–bone regeneration.²⁸⁹ An *in vitro* study showed a gradual decrease in GF concentration due to GF diffusion from one end to the other (PDGF-BB from right to left and vice versa), and the release studies demonstrated a gradual release of GFs over 35 days.²⁸⁹ *In vitro* biological analyses (RT-PCR and Western blot) showed up-regulation of tendon-specific genes (Scx and TNMD) on the right side of the membrane, and bone-specific genes (OCN and RunX2) on the left side of the membrane after both 1 and 2 weeks of ADSC seeding.²⁸⁹

Based on the promising results from this study, an *in vivo* rat study was performed by implanting PCL/Pluronic F127/PDGF-BB (no BMP-2) in a rat infraspinatus model.²⁹⁰ Initial *in vitro* analyses showed a 2-fold difference in the PDGF-BB loading density (276.07 ± 5.49 vs $140.31 \pm 8.68 \text{ ng/membrane}$ for non-immobilized group) and a sustained release profile (~ 42 over 21 days for non-immobilized membranes) for the PCL/F-127-

heparin-PDGF-BB group, compared to PCL or PCL/F127 membranes.²⁹⁰ A biomechanical analysis of the implant showed significant improvements in ultimate stress in the PCL/F127/PDGF-BB group over the rest of the groups, although levels were far lower than normal tendon.²⁹⁰ In addition, histologic analyses of PCL/F127/PDGF-BB membranes showed greater reconnection of neo-tendon tissue to the greater tuberosity and gradual maturation at 4 weeks of repair.²⁹⁰ Furthermore, fibrocartilage tissue formation with oriented collagen fiber was greater in the PCL/F127/PDGF-BB membrane, compared to other groups, suggesting the presence of a suitable environment for rotator cuff regeneration.²⁹⁰

5.3. Fibroblast Growth Factor.

Fibroblast growth factor (bFGF or FGF-2) induces cellular proliferation, thereby promoting collagen synthesis by cells, such as fibroblasts and MSCs.²⁹¹ In a rabbit rotator cuff healing model, the presence of bFGF has been reported to last from post-operative day to 56 days, with peak expression observed at 7 days post surgery, indicating the role of bFGF during both inflammatory and healing phases.²⁹² These results were further corroborated *in vitro*²⁹³ and in rat tendon models,²⁹⁴ which have shown that administration of bFGFs (up to 100 ng) increased proliferation ($P < 0.03$) and collagen type-I and type-III synthesis by tendon fibroblasts after 1 week of bFGF administration.²⁹⁴ Because of these reasons, bFGF administration by topical methods or through scaffolds have been evaluated *in vitro* and *in vivo* for rotator cuff regeneration.

Ide and colleagues investigated the effects of bFGF delivered by fibrin sealant embedded with²⁹⁵ or without an additional ECM scaffold (acellular dermal matrix)²⁹⁶ in a rat supraspinatus model. Histologic analyses of bFGF-treated specimens with no ECM scaffold showed no inflammatory cells and the presence of a few chondrocytes at 4 weeks, facilitating the fibrocartilage formation and subsequent remodeling by 6 weeks.²⁹⁶ In contrast, bFGF-non-treated specimens showed lower cellularity and discontinuity of collagen fibers (compared to bFGF-treated). In addition, histologic and biomechanical analyses showed higher tendon–bone maturation scores (15.8 ± 0.8 for vs 10.6 ± 0.5 for bFGF-non-treated) and tensile strength (6.6 ± 2.0 vs 3.2 ± 0.6 N for bFGF-non-treated) at an earlier time point (2 weeks), but not at later time points (4 and 6 weeks).²⁹⁶

In a subsequent study with an additional ECM scaffold, a reverse in trend was seen in both mechanical and histological evaluation.²⁹⁵ Tendon maturing scores were higher for bFGF-treated groups at 6 and 12 weeks (24.3 ± 1.0 and 26.7 ± 0.8 vs 20.6 ± 2.6 and 25.2 ± 0.5 for bFGF-non-treated), but not at the earlier time point (2 weeks: 11.2 ± 1.7 vs 11.1 ± 0.9 for non-treated; $P = 0.309$)²⁹⁵ A similar trend was also seen in the ultimate force to failure testing, which showed statistical significance at later time points (6 and 12 weeks: 10.2 ± 3.1 and 15.9 ± 1.6 vs 7.2 ± 2.2 and 13.2 ± 2.0 for bFGF-non-treated).²⁹⁵

Like ECM scaffolds, GHS is another scaffold, that has been utilized for administering bFGF in a rat model.²⁹⁷ In a rat supraspinatus defect model study,²⁹⁷ bFGF concentrations were varied and additional experiments such as immunohistochemical analyses, cell number estimations, Sox9 expression, Scx and TNMD mRNA analyses, in addition to biomechanical and histologic analyses, were evaluated.²⁹⁷ Based on histologic and ultimate load scores at 2 weeks, an optimal dosage of 5 μ g was chosen for the *in vivo* study.²⁹⁷ Biomechanical

evaluations of *ex vivo* implants showed higher load to failure and stress to failure values in the bFGF-treated group at both 6- and 12-week time points.²⁹⁷ However, higher stiffness values were observed in the bFGF group at only 6-weeks (7.16 ± 3.28 vs 3.43 ± 1.24 N/mm; $P = 0.005$), but not at 12-weeks (8.98 ± 3.58 vs 6.83 ± 1.25 N/mm; $P = 0.310$). Finally, cross-sectional areas in both treated and non-treated groups were similar at all time points.²⁹⁷ Similarly, higher collagen orientation and total scores were obtained at 4-, 6-, 8-, and 12 weeks from histologic analyses, and higher levels of cells were found to be positive for proliferating cell nuclear antigen (PCNA) indicated gradual tendon tissue regeneration toward that of mature native tissue.²⁹⁷ The gene analysis showed up-regulation of Scx (4–8 weeks) and TNMD (4–12 weeks), further demonstrating the role of bFGF in not only promoting proliferation but also in modulating tendinogenesis.²⁹⁷ These studies demonstrated not only the potential of bFGF for regenerating the rotator cuff, but also the need for optimal delivery technique and timing for rotator cuff regeneration.

Fabricating bFGFs embedded in a core–sheath fashion in a polymeric nanofibrous matrix is another way to deliver the GFs at a sustainable rate for promoting tissue regeneration.²⁹⁸ A comparative *in vitro* study showed that by embedding bFGFs in PLGA nanofibers, faster release and subsequent higher proliferation with large collagen synthesis can be realized, when compared to PLGA/bFGF in a core–sheath fashion.²⁹⁸ Based on the promising results, an additional *in vitro* study was carried out in a biohybrid scaffold consisting of bFGF eluting PLGA nanofibers embedded in a knitted silk scaffold seeded with BMSCs.²⁹⁹ In addition to higher proliferating cell numbers, higher gene expression levels of tendon specific markers (COL1A1, FN, BG) along with higher collagen content and larger failure to load values (82.7 vs 68.2 vs 61.5 N for BMSC/PLGA/bFGF, BMSC/PLGA/no bFGF, and PLGA/bFGF/no BMSCs, respectively) were observed in the FGF-treated/BMSC group at all time points (7 and 14 days).²⁹⁹

Zhao and co-workers performed an *in vitro* and *in vivo* study to evaluate the potential of PLGA/bFGF nanofibers for regenerating rotator cuff with developed entheses, higher collagen orientation, and fibrocartilage formation. Release profiles and subsequent cell proliferation studies revealed sustained release (24 days) and its impact on proliferation (higher at 3 and 5 days over 1 day),¹⁷⁰ consistent with the studies reported by Sahoo et al.^{298,299} Histologic analyses further revealed at 2 weeks post surgery, a gradual resorption with some fibroblasts present in the interstices of the membrane in bFGF-treated group. At 4 and 8 weeks, formation of aligned fibrocartilage tissue was observed, which was largely absent in the bFGF-non-treated group.¹⁷⁰ Consistent with the H&E staining, Safranin-O and Picro Sirius red staining showed significantly higher metachromasia and collagen orientation, respectively, in the bFGF-treated group at all time points (2, 4, and 8 weeks), compared to the other groups (bFGF-non-treated and control).¹⁷⁰ Finally, biomechanics studies of the retrieved explant revealed higher load bearing capability, stiffness, and maximum stress in the bFGF-treated group at later time points (4 and 8 weeks), but not at the early time point (2 weeks).¹⁷⁰ In summary, although bFGF addition caused improvements in cell proliferation and possible mechanical improvements at later time points, addition of other GFs such as PDGF-BB or IGF-1 need to be considered to mitigate inflammation, while simultaneously promoting collagen synthesis at early time points, paving the way for successful rotator cuff regeneration.^{300,301}

5.4. Insulin-like Growth Factor.

IGF-1 is a single-chain polypeptide growth factor that is activated by TGF- β 1 signaling upon injury to the tendon tissue.³⁰² Unlike early-stage GFs such as bFGF or PDGF-BB, high levels of IGF-1 expressions are observed only upon rotator cuff injury.³⁰³ IGF-1 is hypothesized to play a key role in several aspects of rotator cuff healing in the inflammatory and healing phases, as evidenced by high levels of expression between 1 and 14 days of injury (peak between 3 and 5 days).^{292,304} In addition, rotator cuff tear models in rabbits have shown high levels of expression in the proximal and defect regions by vascular endothelial cells and blood cells, respectively. IGF-1 expression was not seen in the distal regions of the torn rotator cuff tendons, however.²⁹² The administration of IGF-1 is known to have impact on maintaining stemness of MSCs,³⁰⁵ cellular proliferation, glycosaminoglycan (GAG) production, ECM synthesis, DNA synthesis, and to decrease swelling leading to increased tissue stiffness.^{291,306} Because of these reasons, several *in vitro* as well as *in vivo* studies have been carried out to understand the effect of IGF-1 in tendon regeneration, and some of these studies are succinctly summarized in an excellent review by Sigal et al.⁸³

Currently, most *in vivo* studies involving IGF-1 administration are intended for regenerating patellar, superficial digital flexor, and flexor digitorum profundus tendons.⁸³ Nevertheless, a few *in vitro* and *in vivo* studies have evaluated the efficacy of IGF-1 for rotator cuff regeneration. For example, a study by Dines et al. compared the effects of IGF-1 and PDGF-BB transfected rat tendon fibroblasts for cell proliferation and collagen synthesis (*in vitro*), and rotator cuff regeneration via delivering transfected cells by a porous PGA membrane *in vivo*.³⁰⁷ While DNA and collagen synthesis by IGF-1-transfected cells were significantly lower compared to PDGF-BB transfected cells at 24- and 48-h time points, biomechanical evaluations (6 weeks of repair) showed significant improvements in load bearing capability in the IGF-1 group, compared to the other groups (PGA+PDGF-BB, Vicryl, Vicryl+PGA).³⁰⁷ This study demonstrated that despite showing lower collagen and DNA synthesis, IGF-1 administration has the potential to improve the biomechanical properties of rotator cuff tissue *in vivo*.³⁰⁷

A major hurdle in the widespread clinical use of GFs, including IGF-1, is the high cost and dosages required along with the long regulatory process for approval.¹⁹⁰ Because of their low cost and FDA approval, insulin, an endogenous anabolic hormone closely resembling IGF-1, is an attractive option for rotator cuff tendon regeneration. Studies have shown that one time treatment of BMSCs (isolated from the proximal humeral head (PHB)) with insulin (10^{-10} M concentration) on BMSCs (isolated from proximal humeral head (PHB)) can cause their differentiation into the tenogenic lineage (COL1A1, COL3A1, TN-C, and decorin).⁸⁵ Subsequent comparison of gene expression profiles of insulin-, bFGF-, and GDF-5-treated BMSCs by RT-PCR showed up-regulation of COL1A1, COL3A1, Scx, and decorin genes in the insulin-treated group, similar to that of the IGF-1.⁸⁵ To further verify, Western blot and immunohistochemical analyses were performed, that showed similar trends as observed by RT-PCR, suggesting that insulin may be an excellent alternative for IGF-1 in rotator cuff regeneration.⁸⁵

A subsequent *in vitro* study revealed the highest expression levels of Scx and COL1A1 (via immunocytochemistry) in an insulin administered group, compared to GDF-5, a well-known tenogenic GF. In addition, low serum (1% vs 10%) also was observed to play a key role in such differentiation.³⁰⁸ Even though these studies demonstrated the potential of IGF-1 and insulin in rotator cuff regeneration, further experiments are warranted, especially in combination with other GFs—as IGF-1 is typically expressed in overlapping expression with other GFs—for their potential in rotator cuff regeneration.⁸³

5.5. Transforming Growth Factor.

The superfamily of TGFs exists in three isoforms, i.e., $\beta 1$, $\beta 2$, and $\beta 3$, with each consisting of 25 kDa homodimers.³⁰⁹ Unlike the other GFs mentioned, TGF was observed via a rabbit rotator cuff healing model to be present during all three phases of wound repair (inflammation, healing, and remodeling) (all 28 days).²⁹² This is supported by studies implicating TGFs as a stimulant and promoter of (1) cell migration,³¹⁰ (2) collagenase and cellular activities,³¹¹ (3) protein–cell interactions and ECM synthesis,³¹² and (4) the interfacial healing process.³¹³ In this study, high expression of TGF- β (along with IGF-1) was observed in the blood cells of the proximal and defect regions of the rotator cuff tendon.²⁹² Consistent with this study, a rat rotator cuff injury model showed the presence of TGF- $\beta 1$ and TGF- $\beta 3$ expression levels (protein and mRNA). TGF- $\beta 1$ gradually increased from time 0 up to 10 days, with a subsequent decrease in the levels,³¹⁴ whereas the protein expression levels of TGF- $\beta 3$ gradually increased from day 3 onward, and highest expression levels were seen at day 28. Additionally, histologic analyses showed localization and absence of TGF- $\beta 1$ (scar tissue) and TGF- $\beta 3$, respectively, at the insertion site, demonstrating the need for modulation of TGF signaling for successful scar-less rotator cuff regeneration.³¹⁴

To investigate the isolated effects of TGF- β signaling in tendon–bone regeneration, Kim et al. evaluated the scar volume, ECM protein synthesis, and biomechanics after administering different doses of either TGF- $\beta 1$ or TGF- $\beta 3$ in a rat supraspinatus model.³¹⁵ Histologic grading based on inflammation, fibrocartilage formation, vascular and fibroblasts proliferation were identical in both groups (TGF- $\beta 1$ or TGF- $\beta 3$) at 1 and 4 weeks.³¹⁵ However, key differences observed were in the ECM protein levels during this period.³¹⁵ At 1 week, levels of collagen type-I and -X and aggrecan were higher, and collagen type-II and -III were lower in TGF- $\beta 3$ group.³¹⁵ At 4 weeks, levels of collagen type-I and -III and aggrecan were lower, and levels of collagen type-II and -X and decorin were higher in TGF- $\beta 3$ group, demonstrating a shift in the trend in ECM protein levels between the two time points.³¹⁵ Despite the differences in key ECM protein levels, trends in collagen orientation, scar tissue (volume), and mechanical properties of the tissues were identical with administration of TGF- $\beta 1$ or TGF- $\beta 3$, illustrating the need for optimal dosage and additional augmentation for optimal healing.³¹⁵

Instead of single-dosage administration, a subsequent study investigated sustained delivery via a heparin-bound TGF- $\beta 3$ embedded in a fibrin hydrogel.³¹⁶ An *in vitro* release kinetics study showed a sustained delivery of TGF- $\beta 3$ over a 10-day period, with over 40% of TGF- $\beta 3$ retained at the end of day 10.³¹⁷ Histologic analyses of *in vivo* explants (rat supraspinatus

model) showed gradual decreases in fibrosis, inflammation, cellularity, proliferation, and vascularity from day 14 to 56; however, fibrocartilage tissue formation was not observed at 56 days.³¹⁶ In addition, biomechanical evaluations revealed higher load values at day 28, but other properties (stiffness, toughness, modulus, stress) were not significant at both 28 and 56 days.³¹⁶

Combining scaffolds with GFs is another potential avenue for enhancing tendon–bone healing. However, two studies that combined osteo-inductive molecules such as BMP-2 (in fibrin matrix)³¹⁸ and calcium phosphate (as injectable matrix)³¹⁹ with TGF- β 3, showed no biomechanical improvements with the addition of TGF- β 3, compared to the controls (BMP-2 or calcium phosphate).^{318–320} Sub-optimal biomechanical properties and lack of fibrocartilage formation were observed with the administration of TGF- β 3,^{315,319,320} therefore demonstrating the need for identifying the optimal TGF- β dosage as well as using appropriate scaffolds for enhanced tendon–bone healing.

5.6. Bone Morphogenetic Proteins and Osteo-Inductive Factors.

Bone morphogenetic proteins (BMPs) belong to the superfamily of TGFs (discussed earlier) that includes TGF- β s and GDFs.^{15,83,197} Within the class of BMPs, several isoforms exist, including BMP-2 and BMP-7 known for their strong osteo inductive potential, and BMPs such as BMP-12, 13, and 14 (also called GDF-7, GDF-6, and GDF-5) known for their non-osteogenic potential (primarily chondrogenic and tenogenic).^{321,322} Due to their potent osteogenic characteristics, the primary intended application of BMPs are in bone regeneration. However, recent research suggests a prominent role of BMP-2 in chondrogenic differentiation via over-expression of Sox9 expressions in MSCs.³²³ In addition, recent chondrogenic media formulations now include the addition of BMP-2, BMP-7, and TGF- β , further demonstrating the pivotal role of BMP-2 in chondrogenesis.³²⁴ Furthermore, being a potent osteogenic inducer places BMP-2 and BMP-7 at a critical cross-roads for tendon–bone healing, which has motivated further research for rotator cuff regeneration.

In one approach, the efficacy of BMP-2 was evaluated *in vivo* in a rabbit model by first administering BMP-2 into the flexor digitorum communis tendon.³²⁵ The ectopic ossicle formed was subsequently implanted into the tibial bone.³²⁵ One month post surgery, biomechanical and histologic analyses showed higher failure to load values (45.6 ± 12.8 vs 26.9 ± 7.9 N for non-BMP-2 group), and formation of fibrocartilage between tendon and bone and an intra-tendinous bone mass in the tibial tunnel for the BMP-2 group.³²⁵ Unlike this study which required two surgical procedures, similar results have been obtained in a rat supraspinatus rotator cuff model with the direct administration of BMP-2 using injectable PEGDA hydrogels.¹⁵⁹ Biomechanical analyses at 4 and 8 weeks post repair showed superior performance of PEGDA/BMP-2 in terms of interface strength, compared to controls at various time points, with values of the PEGDA/BMP-2 group approaching that of uninjured normal rotator cuff tendon.¹⁵⁹

To mitigate the potential of endochondral ossification, BMP-2 is also combined with BMP-7, as BMP-7 is known for regulating the osteogenic differentiation potential of BMP-2.³²⁶ While the activities of BMP-2 and BMP-7 on rotator cuff tenocyte- like cells were similar in terms of cell activity and COLA1 production at low doses, BMP-2 showed

decreased cell activity and COL1A1 production at high dosage (>100 ng/mL). Additionally, when compared between the study groups, BMP-7 performed better than BMP-2, and neither study groups revealed any OCN or Scx production, indicating stemness maintenance of the cells.³²⁷

Administering a cocktail containing osteo-inductive factors is another strategy, which has been evaluated by two separate studies in an ovine model.^{134,328} One approach utilized commercially available bone protein extract from bovine cortical bone (Growth Factor Mixture, Sulzer Biologics), which was subsequently embedded in a collagen matrix, prior to implantation.³²⁹ In the second study, demineralized cancellous bone was fabricated into a 3-D scaffold measuring 3×3×40 mm in blocks.¹³⁴ MRI and biomechanical analyses from the study by Rodeo and co-workers revealed significant bone and soft tissue regeneration and high load to failure values for the GF group at 6 and 12 weeks post repair, compared to the control groups (collagen sponge and suture only controls).³³⁰ Similarly, histologic analyses (MacNeal's tetra chrome and toluidine blue stain) from a Dickerson et al. study showed a gradual transition of the bone-calcified fibrocartilage-cartilage-tendon interface in supraspinatus and infraspinatus tendons, suggesting a potential role of osteogenic factors in rotator cuff tendon healing.¹³⁴

5.7. Platelet-Rich Plasma.

Platelets play a key role in orchestrating a cascade of events in the healing process that begin with the initiation of hemostasis, followed by inflammation, healing, and remodeling, making exogenous administration of platelets attractive for repairing tissues.³³¹ The exogenous application of platelets becomes even more attractive for repairing tissues such as ligaments and tendons, which heal sub optimally forming scar tissue with poor biomechanical characteristics. PRPs contain many of the GFs (TGF- β , bFGF, PDGF, IGF-1, VEGF, etc.) implicated in the rotator cuff tendon healing process.^{312,321} Platelet rich plasma (PRP) is obtained by spinning the whole blood through a centrifugation process, resulting in higher levels of platelets—typically 2×–10×,⁸³ but up to 50×³³²—than that of whole blood.⁸³ This is typically accomplished by varying the spinning process, spin times, number of spins, and further processing of the end product (buffy coat or plasma).³³³ Commercially available PRP-based products vary based on platelet counts, concentrations of erythrocyte and leukocytes, concentrations of cytokines and GFs, delivery methods (gel or injections), and activation of PRP (*ex vivo* by human or bovine thrombin and calcium or *in situ* by collagen).³³⁴ In spite of their obvious potential, widespread applications of PRP-based therapeutics in clinical settings are hampered by variability and non-uniformity, leading to both positive and negative results seen in animal and human studies. To mitigate the variability issues, several classifications have been proposed, such as those based on (1) PAW (platelet amount, activation, and white blood cell) contents;³³⁴ (2) platelet concentrates' sub-classification into pure PRP, leucocyte-rich PRP, pure platelet-rich fibrin, and leucocyte-and-platelet-rich fibrin (L-PRP);³³⁵ and (3) presence of white blood cells and activation/inactivation of PRP (four types)³³⁶.

Despite the obvious differences in PRP formulations, PRP administration has shown promise to stimulate cell proliferation of MSCs,¹³⁸ and tenocytes from rotator cuff tendons

resulting in up-regulation of tendon matrix gene expressions (COL1A1, COL3A1, TN-C, and GAG synthesis).³³⁷ In particular, 1- to 5-fold PRP concentrations were seen to be optimal for improving the proliferation of tendon fibroblasts, with further increases in PRP concentration showing no improvements compared to controls (at 21 days: 468.8% vs 403.8% for no PRP formulation).³³⁸ In addition to low proliferation compared to 1× and 5× PRP formulations, with the exception of 14 days, DNA: GAG ratios were also lower in 10× PRP formulations with values closely resembling that of controls (no PRP) at all time points (1, 3, 7, and 21 days).³³⁸ These results were also observed, when PRP was exposed to chondrocytes and tenocytes in the presence of anti-inflammatory and pro-inflammatory compounds. For example, when PRP, ketorolac, and methyl prednisone were exposed to chondrocytes and tenocytes, a 7-fold increases in cell numbers were observed after 120 h of exposure in PRP alone group.³³⁹ Similar results were also observed when PRP was combined with ketorolac.³³⁹ Even though this effect was substantially lower when combined with methyl prednisone, addition of PRP was found to negate the toxic effects of methyl prednisone.³³⁹

A subsequent study further suggested the phenomenon is due to a decrease in cellular inflammation markers such as E-selectin, vascular cell adhesion molecule, and human leukocyte antigen DR.³⁴⁰ These results were substantiated by additional studies, which showed marked decreases in the anti-inflammatory markers for other joint syndromes (osteo-arthritis).³⁴¹ Furthermore, PRPs have been shown to have bacteriostatic characteristics toward *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epi*), *Propionibacterium acnes* (*P. acnes*), and methicillin-resistant *Staphylococcus aureus* (MRSA).³⁴² In summary, the administration of PRP is a potential route to impart anti-bacterial and anti-inflammatory activities, while simultaneously stimulating proliferation and ECM synthesis by cells.^{339,342}

PRP administrations *in vivo* have resulted in significant improvement in terms of pain reduction,^{343–347} biomechanics (range of motion),^{345,346,348} low re-tears,^{130,344} qualitative scores (VAS, UCLA, ASES, Constant, etc.)^{349,350} and by radiographic and MRI analyses^{332,343} in some human clinical trials. In addition, PRP has been shown to be safe with infection rates similar to that of controls.³⁵¹ However, these results need to be taken with caution as several, if not an equal number of studies have shown no significant pain relief,³⁵² quality of life and range of motion,^{352,353} re-tear rates,^{354–356} shoulder scores,^{348,352,354–358} or MRI analyses,^{348,354,355,357,358} with the administration of PRPs. In addition, two meta-analyses on human trials for rotator cuff regeneration have shown no significance in terms of re-tear rates, strength regain and other clinical outcomes with PRP administration.^{359,360} To negate the differences across the studies, more animal studies are being explored to standardize surgical procedures,^{361–363} which could potentially delineate the potential of PRPs in regeneration rotator cuff.

5.8. Small Molecules and Pharmacologic Agents.

Small molecules are low-molecular weight (~<1 kDa) bioactive signaling molecules that can permeate the cell membrane and up-/down-regulate signaling pathways involved in tissue regeneration.¹⁹⁰ Key advantages with small molecules include their high cell permeability,

high stability, low immunogenicity and independence from protein conformation, in addition to their potential low cost and low dosages requirements, making them attractive for use in tissue regeneration.¹⁹⁰ In the musculoskeletal system, several molecules such as statins, rapamycin, phenamil, 4-Bnz-cAMP have been studied for their potential in bone regeneration.¹⁴ Likewise, kartogenin is a well-known chondrogenic promoter, leading to the formation of cartilage tissue in some studies,³⁶⁴ including the tendon–bone junction.³⁶⁵ Similarly, several small molecules such as tazarotene (a retinoic acid receptor agonists);³⁶⁶ miR-29a;³⁶⁷ and modulators of BMP-2 (quinacrine and niclosamide) and TGF (vorinostat and hycanthone)³⁶⁸ have been identified for their potential role in tendon/rotator cuff regeneration. These small molecule function uniquely. For example, tazarotene functions by preventing spontaneous differentiation of MSCs, and by up-regulating Scx expression.³⁶⁶ Likewise, miR-29a functions by regulating IL-33 expression, a key expression molecule for transition from type-III collagen synthesis.³⁶⁷ As torn rotator cuff tissue typically leads to fibrosis, fatty infiltration, and muscle degeneration, three small molecules, i.e., SB431542,³⁶⁹ nilotinib,³⁷⁰ and imatinib,³⁷¹ have been implicated for causing apoptosis of fibro-/adipogenic progenitors via disrupting TGF- β and PDGF-receptor signaling pathways, respectively, thereby demonstrating wide utility of small molecules in rotator cuff regeneration.

Common NSAIDs administered post surgery also have an impact on tissue healing via their effect on matrix metal-loproteinase (MMP) activity. For example, tetracycline, a commonly used antibiotic, administered to rats post surgery showed increases in metachromasia and collagen orientation, resulting in high load to failure values.³⁷² Similarly, administration of licoferone to rats showed drastic decreases in the levels of inflammatory 5-Lox, Cox-1, and Cox-2 enzymes resulting in improved mechanical properties (load, stress, and stiffness; $P < 0.05$), compared to controls.³⁷³ Conversely, several antibiotics and NSAIDs such as fluoroquinolone,³⁷⁴ methylprednisolone,³⁷⁵ betamethasone,³⁷⁵ meloxicam,³⁷⁶ celecoxib,³⁷⁷ valdexicob,³⁷⁷ and piroxicam,³⁷⁷ acetaminophen,³⁷⁷ have shown detrimental effects in varying degrees at normal dosages to healing tissue, resulting in suboptimal structural characteristics and biomechanics in the rotator cuff tendon. These studies thus suggest the need for careful selection and timing of drug administration for mitigating any detrimental effects of pharmacologic agents on the healing of rotator cuff tissue.

6. MECHANICAL STIMULATION

In ligament/tendon regeneration, mechanical loading affects the healing process in numerous ways.³⁷⁸ For example, applying uniaxial strain has been found to induce cell alignment and ECM production along the direction of applied mechanical loading.^{379,380} In addition, mechanical loading is known to cause tenogenic differentiation of MSCs, proliferation of cells and accelerated ECM synthesis, which are considered to be chief attributes of the ligament/tendon tissue remodeling process.^{381–384} This is due to the active response of the tendon tissue to mechanical loading, as observed in physical training or physical activity.^{385,386}

For instance, a biomechanical evaluation of mechanically stimulated (4% strain for five minutes/8h) stem-cell constructs showed a 2.5 times higher stiffness *in vitro* (2 weeks),

and in a rabbit model, histologic analyses showed excellent cellular alignment and staining for decorin and COL1A1, and moderate staining for fibronectin and COL3A1 expressions. The study further showed significant healing of patellar tendon tissue at a 12 week time point.³⁸⁷ To isolate the effects of scaffold composition and architecture, various scaffold types (gels vs sponges) with differing architectures (long vs short) were evaluated. Longer constructs showed higher linear stiffness in all study groups (gels vs sponges and stimulated vs non-stimulated), compared to shorter constructs. Mechanical stimulation has substantial effects in improving the linear stiffness of shorter cell–sponge constructs ($P = 0.003$), compared to longer constructs ($P = 0.03$), indicating the effects of mechanical stimulation may have interactive effects with other factors such as scaffold architecture.³⁸⁸ To further isolate scaffold stiffness and mechanical stimulation, scaffolds with varying stiffness (dehydrothermal cross-linking) were evaluated for the biomechanics (*in vitro*) and histology of regenerated tendon tissue (*in vivo*). While the histology did not show any significant improvements in terms of tenocyte density or cellular alignment in both scaffolds (the stiffness of the constructs without cross-linking improved significantly, while no such effect was seen in scaffolds cross-linked by ADHT).³⁸⁹

Although one study³⁸⁸ evaluated the effect of mechanical stimulation on scaffold constructs (sponges vs gels), the effect of mechanical stimulation of biomaterials has been largely understudied. Recently, the effects of mechanical stimulation on linear modulus and stiffness were evaluated *in vitro*, and *in vivo* tenogenic differentiation and ECM proteins levels were evaluated in collagen- and fibrin-based constructs.³⁹⁰ Biomechanical evaluations of fibrin- and collagen-based scaffolds showed linear increases in stiffness over studied time points (6, 10, and 14 days) under static and mechanically stimulated conditions. However, no differences were observed between static and stimulated groups at all studied time points for both scaffolds, except for the collagen scaffold at day 10.³⁹⁰ Interestingly, linear increases in tenogenesis were observed in fibrin scaffolds via tendon differentiation markers (scx, tn-c, Tnmd, Col1a1, Col3a1, Fmod) at increasing time points. These results were further corroborated by immunostaining analyses, which showed the presence and alignment of ECM proteins across fibrin scaffolds compared to collagen scaffolds. In addition, mechanical stimulation did not show any significant effect on the expression of these proteins.³⁹⁰

Although the effect of mechanical stimulation has been known for about a decade now, the underlying molecular mechanisms responsible for tissue regeneration, and ways to effectively utilize them in tissue regeneration are slowly being elucidated in the current decade. The effect of IGF-1, an anabolic GF discussed before, on tendon regeneration is well known. One reason why IGF-1 is important in tendon regeneration is because of its Eb-isoform, mechano-growth factor (MGF), which potentially translates mechanical force into cellular transformations.³⁹¹ A key question regarding the optimal mechanical loading on gene expression levels was recently elucidated by a study in which various levels of strain (moderate treadmill training (MTR) and intensive treadmill training (ITR)) were evaluated.³⁹¹ Under both levels of training, MGF was almost 10-fold higher than the control conditions, and in addition, the growth of patellar tendon- and Achilles tendon stem cells were substantially higher in these conditions. However, key differences in loading types on cell types (tendon stem cells and tenocytes) lay in terms of tenogenic and non-

tenogenic differentiation observed by specific genes. In general, animals that underwent ITR showed substantial increases in both tenogenic and non-tenogenic differentiation markers (adipocytes, chondrocytes, and osteo-blasts).³⁹¹ Similarly, increasing mechanical stretching from 4% to 8% also showed similar effects in terms of up-regulation of both tenogenic as well as non-tenogenic gene expression levels. These results indicate excessive loading of tendons leading to “chronic microinjuries”, resulting in the pathogenesis of the tendinopathy.³⁹¹

In addition to the well-known effects of IGF-1 (or its isoform MGF), numerous other GFs such as TGF- β 1, CTGF, IL-6, IL-8, FGF, NO, VEGF, and PDGF, have shown both positive and detrimental effects toward mechanical loading.^{392,393} For example, mechanical loading of TDSCs have shown an up-regulation of BMP-2 gene expression, indicating the possibility of ectopic bone formation.³⁹⁴ Likewise, another study demonstrated fewer apoptotic cells, higher collagen orientation, down-regulation of inflammatory markers by simple immobilization of animals, compared to delayed and immediate post-operative mechanical loading.³⁹⁵ On the other hand, mechanical loading of animals post surgery has showed the presence of numerous apoptotic cells (~20%), compared to unloaded animals (<1%), evidenced by abnormal nuclear morphology and cleaved caspase-3 staining.³⁹⁶ From these studies, it is therefore obvious that mechanical loading of scaffolds before surgery or post-surgical external stimulation could have significantly contrasting results. Given the fact that most of these studies were based on tendon tissues such as patellar-, and Achilles tendons, care must be taken to extrapolate these studies for RC tendons. Also, the effect of scaffold constructs must be evaluated in conjunction with other GFs, and tested and evaluated *in vivo* for synergistic effects toward RC regeneration.

7. CLINICAL TRIALS ON ROTATOR CUFF REGENERATION

According to clinicaltrials.gov, currently, there are 289 clinical trials underway or recently completed for treating/repairing rotator cuff lesions. Out of 289 clinical trials, ~62% of clinical trials are being carried out in the western hemisphere (North and South America) and the rest in eastern hemisphere (Asia, Africa, Europe, Australasia, and the Middle East) (Figure 4A). Among these clinical trials, the majority of the trials investigate surgical procedures and techniques (~40%), followed by studies investigating the effects of post-surgical rehabilitation and exercise regimen (~17%) and imaging modalities (~5%) that can facilitate/improve our understanding of rotator cuff lesions (Figure 4B).

Scaffolds currently being studied for rotator cuff regeneration include InSpace (PLCL balloon); SPARC: Shoulder PAtch (composition unknown, clinical trials identifier: [NCT02049684](https://clinicaltrials.gov/ct2/show/study/NCT02049684)); Augment (bovine collagen matrix+PDGF-BB); OrthoAdapt (equine pericardium patch); Conexa (porcine acellular dermal mesh); platelet-rich fibrin membrane; Arthrex DX Reinforcement Matrix (acellular ECM); human dermal allograft; polyester patch (composition unknown, clinical trials identifier: [NCT02976285](https://clinicaltrials.gov/ct2/show/study/NCT02976285)); BioFiber (P4HB mesh); Rotation Medical Rotator Cuff System (collagen bio-inductive implant); and human umbilical cord allograft (MiMedx Group, Inc.).

A major segment of biologics being studied involves PRP administration. Current studies focus on the (1) timing of PRP injections (pre, post, or no repair); (2) implantation of PRFM scaffold; (3) administration type (suturing PRP with gels or native tissue); (4) sequela of PRP administration; and (5) administering PRP in combination with an external ECM device (for example, Arthrex DX Reinforcement Matrix) for rotator cuff tendon healing.

In addition to PRP as a GF source: PDGF-BB, and BMP-65S (composition unknown; clinical trial identifier: [NCT00936559](#)) in a collagen matrix are the two GFs that have been recently evaluated, and currently no clinical trials are being carried out investigating GFs for rotator cuff regeneration at the time of our Review. Besides GFs and PRP, autologous BMSCs and ADSCs are also being studied for rotator cuff regeneration, with a notable product being Regenexx^{SD} for pain relief (clinical trials identifier: [NCT01788683](#)).

Challenges in Rotator Cuff Tissue Regeneration.

Current strategies to repair/regenerate rotator cuff tissue predominantly focus on primary surgical care along with augmentation by ECM grafts. In addition to poor biomechanical outcomes and structural composition differences to the native rotator cuff tissue, augmentation by porcine grafts (SIS) has also shown high rejection rates in some studies, necessitating other strategies such as scaffold-, GF-, and stem-cell-based approaches for rotator cuff regeneration.

Even though all the mentioned strategies demonstrate superior outcomes compared to primary surgery techniques, successful regeneration in terms of load bearing capability, stiffness, and toughness, and structural characteristics and biochemical composition remain elusive. Given the fact that rotator cuff tissue has a heterogeneous structure, it is conceivable that a multi-phasic scaffold would be more appropriate for rotator cuff regeneration. However, current scaffolds being investigated are relatively simple structures (micro and nanofibers, or a combination thereof) with no heterogeneity to account for complexity, as seen in the native tissue. Similarly, individuals or a group of few GFs are being investigated for their potential role in regenerating tendons. However, multiple GFs are expressed in sequential/overlapping fashion with high temporo-spatial precision, along with the presence of various cells during the tissue regeneration process. In addition, *in vivo* studies clearly demonstrate the need for optimizing the delivery technique and dosages for improving their role in tissue regeneration. Stem cells, in particular, those present in proximity to the tissue, provide excellent opportunities via immunomodulatory, multi-differential, and proliferative potential in tissue regeneration. Nevertheless, stem cell-based therapeutics also requires biochemical cues in temporo-spatial precision for differentiation into the osteo-, chondro-, and tenogenic lineages, required for rotator cuff regeneration.

The emergence of Regenerative Engineering^{397,398} as a field, adapting cues from conventional tissue engineering, advanced material science, stem cell technology, and developmental biology, along with knowledge from clinical translation, is one potential approach for successful rotator cuff regeneration. By such convergence of technologies, it is conceived that advanced biomaterials-based scaffolds would be fabricated with geometry, architecture, and biomechanical properties relevant to the native rotator cuff tissue. In addition, advanced scaffolds are also expected to provide biochemical cues with high

temporal-spatial precision, facilitating proliferation and differentiation of MSCs, leading to the formation of neo-tissue with structural, biochemical, and biomechanical characteristics similar to that of native tissue. Furthermore, with the advent of advanced scaffolds mimicking the mechanical properties of the developing embryo, along with subsequent regulation of differentiation toward mature tendon tissue, we see new paradigms for regeneration.^{399,400} We hypothesize these new Regenerative Engineering technologies may ultimately lead to better more successful outcomes for rotator cuff regeneration.

CONCLUSION

Sub-optimal surgical outcomes in terms of re-tear rates, poor biomechanics, and structural composition, commonly seen in rotator cuff repair, necessitate the investigation of alternate, novel strategies for addressing the potential drawbacks associated with primary techniques. Approaches include evaluation of stem-cell, scaffold-, and growth factor-based therapeutics for rotator cuff regeneration in the setting of physical forces. A key advantage in utilizing MSCs is their capability to undergo tissue specific differentiation and superior proliferative capability. Likewise, native ECM scaffold-based approach provides biochemical cues, facilitating tissue in-growth. Contrastingly, synthetic scaffold-based approach provides mechanical augmentation, and in addition, offer versatility in their capability to be fabricated into diverse structures (nanofibers, microfibrillar, porous sheets), increasing their appeal for tissue regeneration. Newer approaches using these materials work to guide regeneration of stem cells. GFs play a key role in native tissue healing process by actively participating in inflammation, healing, and the remodeling processes. The key aim of GF-based therapeutics is therefore to recapitulate the natural healing process, resulting in the regeneration of tissue lesions/tears. *In vivo*, success also depends on the proper biomechanical environment and in clinical surgical technique. In this summary, we have described state-of-the-art strategies based on stem cells, scaffolds, GFs, use of physical forces, and clinical translation currently utilized for rotator cuff tissue regeneration. We believe these strategies, which we term collectively Regenerative Engineering approaches hold promise for the future of tissue regeneration.

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ABBREVIATIONS

GF

growth factor

SIS

subintestinal submucosa

GJ

GraftJacket

P4HB

poly(4-hydroxybutyrate)

PLA

poly(L-lactic acid)

PG

proteoglycan

BG

biglycan

ACAN

aggrecan

TGF- β

transforming growth factor- β

EGF

epidermal growth factor

FGF- β (bFGF)

basic fibroblast growth factor

G-CSF

granulocyte colony stimulating factor

VEGF

vascular endothelial growth factor

FN

fibronectin

TN-C

tenascin-C

ECM

extracellular matrix

BMSC

bone marrow-derived mesenchymal stem cell

cBMA

concentrated bone marrow aspirate

ACL

anterior cruciate ligament

VAS

visual analog scales

RC

rotator cuff

FACS

fluorescence-activated cell sorting

GFP

green fluorescent protein

TDSC

tendon-derived stem cell

BMS

bone marrow stimulation

HSC

hematopoietic stem cell

PRP

platelet-rich plasma

MRI

magnetic resonance imaging

UCLA

University of California, Los Angeles

DASH

disabilities of the arm, shoulder, and hand

NPS

numerical pain scale

PDGF-BB

platelet-derived growth factor

GDF-5

growth and differentiation factor-5

BMP-12

bone morphogenetic protein-12

RT-PCR

real-time polymerase chain reaction

Scx

scleraxis

ADSC

adipose-derived mesenchymal stem cell

BMP-13

bone morphogenetic protein-13

COL1A1

collagen type-I (gene/protein)

COL3A1

collagen type-III (gene/protein)

BMAC-PRPs

mixture of bone marrow aspirate/platelet-rich plasma

TSG-6

tumor necrosis factor α -stimulated gene/protein-6

EGR1

zinc finger transcription factor early growth response-1

IM

intra-muscular injection

EF

epimuscular fat

SDSC

synovium-derived mesenchymal stem cell

PLSC

placenta-derived mesenchymal stem cell

PRSC

periosteum-derived mesenchymal stem cell

CTPSC

connective tissue progenitor stem cell

MDSC

muscle-derived satellite/mesenchymal stem cell

TNMD

tenomodulin

LHB

long head of the biceps muscle

SSP

supraspinatus muscle

MMP-1

matrix metalloproteinase-1

MMP-2

matrix metalloproteinase-2

SASC

subacromial bursa-derived mesenchymal stem cell

BMP-7

bone morphogenetic protein-7

TPI

total pressure index

H&E

hematoxylin and eosin

ALP

alkaline phosphatase

PST

pulsed signal therapy

UCBSC

umbilical cord blood-derived mesenchymal stem cell

PVSC

perivascular stem cell

OR

operating room

GHS

gelatin hydrogel sheet

qPCR

quantitative polymerase chain reaction

HA

hyaluronic acid

PEGDA

poly(ethylene glycol) diacrylates

PPC

periosteal-derived progenitor cell

PTFE

poly(tetrafluoroethylene)

PET

poly(ethylene terephthalate)

ePTFE

expandable poly(tetrafluoroethylene)

ROM

range of motion

SF-12

short form-12

PGA

poly(glycolic acid)

PLGA

poly(lactide-*co*-glycolide)

PCL

poly(ϵ -caprolactone)

PLCL

poly(lactide-*co*-caprolactone)

FBR

foreign body reaction

OCN

osteocalcin

PNEA-mPh

poly ((ethylalanato)₁(*p*-methylphenoxy)₁) phosphazene

PETG

glycol-modified poly(ethylene terephthalate)

COMTS

composites of multi-layered tendon slices

OFM

ovine forestomach

CICP

type-I C-terminal collagen pro-peptide

ASES

American Shoulder and Elbow Surgeons

PDO

polydioxanone

OPLA

open-cell poly(L-lactic acid) sheet

col-spo

collagen sponge

JOA

Japanese Orthopaedic Association

FL

fascia lata

PEO

poly(ethylene oxide)

PCNA

proliferating cell nuclear antigen

GAG

glycosaminoglycan

PHB

proximal humeral head

TGF- β 3transforming growth factor- β 3***S. aureus****Staphylococcus aureus****S. epi****Staphylococcus epidermidis****P. acnes****Propionibacterium acnes***MRSA**methicillin-resistant *Staphylococcus aureus***COX**

cyclo-oxygenase enzymes

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Figure 1.

Ultramicrostructure, transition zones, and biomechanics of the rotator cuff tendon tissue. (A) Hierarchical structure formation in the tendon tissue, beginning with the self-assembly of procollagen into collagen fibrils with a triple-helical structure. The collagen fibrils in turn bundle together forming collagen fibers, which are the key component of tendon fascicle. The tendon fibroblasts and tenocytes reside between the collagen fibers. The parallel aligned fascicles are wrapped by a connective tissue (endotenon), which contains blood vessels, nerves and lymphatics. The clusters of fascicles are in turn encapsulated by another connective tissue layer, epitenon, resulting in the formation of tendon tissue. (B) collagen type varies gradually, and in addition, the collagen fiber orientation varies gradually from highly oriented (in tendon) to randomly oriented (in bone) with intermittent presence of oriented fibers. (C) Biomechanical (stress–strain curve) behavior of the tendon tissue. Upon mechanical loading, uncrimping (I) of the collagen fibrils takes place, leading to the realignment (II) of those fibrils, evidenced by decrease in the variance in the distribution of fiber angles. During the uncrimping to realignment phase, tendon tissue shows high elongation (therefore low stiffness and modulus); however, upon realignment it transitions into a high modulus and stiff tissue (linear region in stress–strain curve). Finally, upon further mechanical loading, the tendon tissue abruptly fails leading to the loss of

fibrillar structure. Panels A–C reproduced with permission from ref 27, ref 49, and ref 52, respectively. Copyright 2014. Wiley Periodicals Inc., 2009 Elsevier Ltd., 2013 Elsevier Ltd.

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Figure 2.

Applying MSCs in a rotator cuff tear leads to the rotator cuff tendon regeneration. (A) Schematic of a rotator cuff tear, where H represents the tear size in the coronal plane parallel to the tendon tissue, and L represents the larger ends of the tear on the foot print. From the H and L values (2.5 and 3 cm, respectively), an effective tear area of 3.75 cm^2 ($2.5 \times 3/2$) can be obtained. (B) Enthesis (fibrocartilage) formation between the bone and tendon tissues, when no cells, chondrocytes, and MSCs were injected into the rotator cuff tear. Although chondrocytes led to a larger secretion of COL2A1 matrix at the earlier time point (15 days), MSCs secreted homogeneous COL2A1 and larger GAG contents at later time points, leading to successful enthesis formation. Modulating the gene expression of MSCs by exposing to GFs is one approach to enhance the potential of neo-tissue formation. (C–G) Insulin, an anabolic growth hormone was exposed to BMSCs, and tendon-related gene expression was studied. Insulin exhibited similar or superior effects when compared

to most GFs studied. For example, COL1A1, decorin, and Scx expressions in insulin group were superior compared to all groups, except bFGF. Likewise, TN-C expressions were higher in IGF-1 and bFGF group, compared to the insulin group. Panels A, B, and C-G are reproduced with permission from refs 80 ref,⁷⁹ ref,¹⁰⁵ respectively. Copyright 2014 Springer Berlin Heidelberg, 2010 Nourissat G, Diop A, Maurel N, Salvat C, Dumont S. Pigenet A, Gosset M, Houard X, Berenbaum F. 2011 Elsevier Ltd.

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Figure 3.

Engineered matrices with stem cell niches aids in regenerating rotator cuff tissues. (A) Masson trichrome stained images of matrices retrieved after 12 weeks of implantation in a rat rotator cuff model (infrapinatus). In the case of native initially fabricated PCL nanofibers, and subsequently functionalized with polyphosphazene poly((ethyl alanato)₁(*p*-methyl phenoxy)₁) phosphazene (PNEA-mPh) to improve hydrophilicity. The surface functionalized matrices were then seeded with BMSCs and cultured for 2 days, upon which the cell-laden scaffolds were implanted in a rat rotator cuff integrative augmentation model. Masson trichrome analyses at 12 weeks showed gradual transition of aligned parallel collagenous tissue to the bone with a mid-substance in BMSC-laden constructs (part IV) and native controls (non-operated) (Fig 3A tendon (I) and tissue regenerated with BMSC-laden engineered matrices (IV), a more homogeneous transition from tendon to tissue is observed, whereas in repair only (II) and matrix augmented repair with no cells (III), an

abrupt transition is seen, indicating higher possibility of scar tissue formation than tissue regeneration. (B) Morphology of the native tendon tissue (I), suture only repair (II), augment only (III), and cell-laden constructs (IV), obtained by H&E staining after 12 weeks of *in vivo* implantation. H&E-stained images shows sparse population of elongated tenocytes along parallel-oriented collagen fibers in the native tendon tissue (I); similarly, the cell-laden constructs (IV) also show higher fiber orientation and cell morphology. In contrast, both the suture repair only and augmentation scaffolds lead to disoriented matrix with overpopulation of cells with rounded morphology, consistent with the Masson trichrome staining. (C,D) Quantified ratios of collagen type-I:collagen type-III and birefringence values from polarized optical microscopy. Consistent with Masson trichrome and H&E staining, higher collagen type-I:collagen type-III ratios and birefringence values (indicator of collagen orientation) in native tendon and cell-laden constructs groups suggests the regenerated tissue is morphologically and biochemically similar to that of native tendon tissue, whereas both suture only and augmentation only groups lead to regeneration that lacks morphological and biochemical features of native tendon tissue. Reproduced with permission from ref.²³⁶ Copyright 2017 Peach SM, Ramos DM, James R, Morozowich NL, Mazzocca AD, Doty SB, Allcock HR, Kumbar SG, Laurencin CT.

Figure 4.

Ongoing clinical trials studying various aspects of rotator cuff regeneration. Worldwide statistics indicate over two-thirds of clinical trials are being conducted in the developed world (Europe and North America), followed by developing world (Asia and South America). In addition, the pie chart demonstrates surgical techniques and procedures, closely followed by non-surgical techniques, to be the most widely studied aspects in rotator cuff regeneration. The pie chart further demonstrates that ~20% of the research is being conducted on various aspects of biologic augmentation (scaffolds, PRPs, stem cells). The data are accurate as of April 10, 2017. Figures adapted and modified with permission from clinicaltrials.gov, 2017.

Table 1.
Some Biologic Augmentation Devices Currently Used for Treating Rotator Cuff Lesions

scaffold	manufacturer	origin and structure	manufacturer's claims of role in tissue healing ^a
Genesys CrossFT	ConMed	sutures (4.5, 5.5, and 6.5 mm diameter) coated with tricalcium phosphate	Maximizes cortical and cancellous fixation. In addition, provides reliable fixation and enables the bone-tendon healing.
Allopatch HD	ConMed	hydrated and dehydrated acellularized human dermal and fascia lata ECM with variable thicknesses	Allopatch, derived from human acellularized tissue, preserves and maintains the natural biomechanical, biochemical, and matrix properties, thereby augmenting the rotator cuff regeneration.
Dermaspan	Biomet	acellularized human dermal ECM with variable thicknesses	Compared to xenografts, reduced graft rejection and inflammatory response. In addition, enhances the tissue reinforcement and graft procedures.
Zimmer collagen repair patch	Zimmer	cross-linked decellularized porcine dermal tissue, available as 1.5 mm thick films	The chemically cross-linked ECM is resistant to enzymatic degradation and has long residence time. In addition, the patch provides strong reinforcement, is durable, and is easy to handle during the surgical procedure. Moreover, the patch facilitates infiltration of fibroblasts and graft integration with the tissue.
ArthroFLEX	Arthrex	acellularized human dermal ECM with a thickness of 1–3 mm	The customized patented process removes donor DNA completely, without removing collagen, elastins, and GFs. As a result, the native biochemical properties of the scaffolds are retained, thereby facilitating the rotator cuff healing.
Conexa	Tornier	decellularized porcine dermal tissue with length and widths ranging from 2 and 6 to 4 and 10 cm, respectively	Higher sterility of the product is guaranteed (10^{-6}) while simultaneously providing key matrix components. As a non-cross-linked material, the scaffold supports rapid cell population and revascularization.
Restore	Depuy Orthopaedics	decellularized porcine SIS matrix (circular) with thickness of 0.8–1 mm and diameter of 63 mm	Capable of repairing more than one tendon in the rotator cuff tissue. The scaffold provides sufficient strength to the tissue, facilitating regeneration and integration of the tissue in the site.
OrthoAdapt	Pegasus Biologics	cross-linked equine pericardium patches 4 × 5 cm (length and width)	Highly cross-linked scaffold provides high mechanical properties, facilitating controlled remodeling and integration with the tissue.
TissueMend	Stryker Orthopaedics	non-denatured fetal bovine dermal matrix available in sizes of 3 × 3, 4 × 4, 5 × 6, and 6 × 10 cm	Acellular collagen fibers are composed of type-I collagen and type-III collagen, similar to that of native tissue. In addition, removal of cells or their components does not have an effect on the native structure of the collagens.
Integra reinforcement matrix	Integra	acellular porcine dermal matrix in sizes 4 × 7 and 5 × 10 cm	The Integra matrix provides high tensile strength, facilitating soft tissue healing, in addition to preventing tearing of the rotator cuff tissue.
GraftJacket (GJ)	Wright Medical	acellular human dermal matrix (non-mesh) with thickness ranging from 0.5 to 2 mm	Due to the positive recognition of the scaffolds by the body, low inflammatory and foreign body responses are observed. In addition, fragments of elastins are seen upon healing, indicating formation of cartilage tissue (in the enthesis).
Bio-Blanket	Kensey Nash Corp. (now Royal DSM)	acellular bovine hide collagen with length, width, and thickness ranging from 1 to 10, 1 to 10, and 0.5 to 5 cm, respectively	Through a patented process, collagen sheets are obtained possessing sufficient mechanical properties, which facilitates the reinforcement and repair of soft tissues.
Artelon	Artelon	knitted polyurethane urea mesh comprised of resorbable and non-resorbable fractions	The scaffold provides long-lasting mechanical strength for many years, facilitating integration of the tissue. Non-enzymatic degradation of the scaffold permits predicted degradation behavior irrespective of the patient's age or physiology. Likewise, large mechanical loading permits fibroblasts infiltration and collagen synthesis.
Biofiber	Tornier	P4HB fibrous scaffold	The scaffold reinforces the suture-tendon interface while stimulating the tendon repairs. As the metabolites of P4HB are non-toxic, cell-friendly environment facilitates rapid integration with the site.

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scaffold	manufacturer	origin and structure	manufacturer's claims of role in tissue healing ^a	
			With long degrading P4HB, long-term mechanical support is assured, with collagen coating facilitating tissue in-growth. As the mechanical integrity is maintained, the scaffold facilitates natural regeneration of tendon–bone attachment with normal joint mechanics.	Biodegradable PLA mesh has mechanical properties similar to those of human tendons. Due to high suture retention strength, X-repair remains in place and shares the load typically encountered by the tendon. In addition, as the graft retains over 90% of the mechanical strength after 12 months of implantation, it provides mechanical augmentation to the repair site.
Biofiber-CB	Tornier	type-I collagen-coated P4HB scaffolds		
X-repair	Synthasome	PLA woven mesh		

^a Adapted from manufacturers' product manuals.

Table 2.

List of Cell Sources Reported for Rotator Cuff Regeneration

cells	experiments	conclusion	ref
muscle-derived satellite/stem cells (MDSCs)	<i>in vitro</i> and <i>in vivo</i> (rat model, 21 days)	<i>In vitro</i> characterization of the purified MDSCs showed lack of vimentin expression but demonstrated up-regulation of β -galactosidase gene <i>in vitro</i> at 3 weeks. The cell morphology transformed into spindle-shaped and was well integrated with the tendon ECM <i>in vivo</i> .	122
MDSCs	<i>in vitro</i> and <i>in vivo</i> (mice, 1 week)	<i>In vitro</i> experiments showed up-regulation of neural cell adhesion molecule and HSC marker (CD45 and CD31) expressions in the MDSCs. The proliferative capabilities of the MDSCs were significantly affected by the tendon tear; in particular, the lowest proliferation was observed in partial tears. Although the proliferative potential was lower, the MDSCs ably fused with regenerating fibers.	123
perivascular stem cells (PVSCs)	<i>in vivo</i> (immunodeficient mice, 6 weeks)	The PVSCs obtained from liposarcoma procedure were sorted by FACS into pericytes and adventitial positive cells. The articular injection of pericytes resulted in lower fibrosis and fatty infiltration.	125
umbilical cord blood-derived stem cells (UCBSCs)	<i>in vivo</i> (rabbit model, 4 weeks)	Ultrasound-guided injection of human UCBSCs in rabbit model demonstrated 7 out of 10 complete subscapularis tears turning into partial thickness tears, while 3 full tears remained unchanged. Histologic analyses further showed collagen fiber regeneration along the muscle fibers. Animals injected with UCBSCs were able to walk for longer distances at high rates of speed, compared to the other groups.	124
adipose-derived stem cells (ADSCs)	<i>in vivo</i> (canine model) in conjunction with PRP	Cross-sectional area of the treated group decreased with the ADSC-PRP administration, and at 90 days post surgery, no statistical difference was observed between contralateral rotator cuff and injured rotator cuff. Likewise, gait analysis showed a decrease in the total pressure index (TPI) after the ADSC-PRP administration.	128
periosteum-derived stem cells (PRSCs)	<i>in vivo</i> (rabbit model, 12 weeks)	The Masson's trichrome and Hematoxylin and Eosin (H&E) staining showed gradual progression of fibrous tissue (at 4 weeks) to mineralization (8 weeks) to more mature tendon-bone lining with mature fibrocartilage (12 weeks) in the periosteum-treated group. In addition, radiographs showed new bone formation at 12 weeks in the periosteum-treated group.	127
tendon-derived stem cells (TDSCs)	<i>in vitro</i> (Lipogems ^a -induced proliferation and differentiation of TDSCs)	After 48 h of culturing TDSCs, Lipogems-treated cells showed significant increases (24%, 9.7%, and 5%) in proliferation, with no differences in cell apoptosis, compared to untreated TDSCs. Similarly, cell phenotype and stem cell marker experiments showed no differences between Lipogems-treated and untreated TDSCs. However, RT-PCR expression showed ~1.3-fold up-regulation of VEGF expressions in the Lipogems-treated group.	129
connective tissue progenitor stem cells (CTPSCs)	<i>in vitro</i> (development of new isolation procedure)	After 7 days of culturing, CTPSCs obtained from operating room setting showed similar morphologies compared to traditional isolation procedures. In addition, MSCs obtained from all procedures showed similar proliferation (colony forming units) trends. Finally, the CTPSCs that underwent 5 min of fractionation showed higher levels of ALP, COL1A1, and bone sialoprotein expressions, compared to the other groups (30 min and 24 h) in osteogenic media.	105
TDSCs	<i>in vitro</i> study of progenitor cells obtained from torn rotator cuff tendons	FACS analyses of TDSCs were positive for commonly used MSC markers (CD29, CD44, CD105, and CD166) and negative for HSC markers (CD14, CD34, and CD45). When cultured under osteogenic, adipogenic, and chondrogenic conditions, TDSCs differentiated along those lineages.	47
subacromial bursa stem cells (SASCs)	<i>in vitro</i> comparison of SASCs and BMSCs	At early time points (3 days), SASCs showed higher proliferative potential, while the reverse was true at later time points (days 7–17). While minor variations were found in cells that were positive (or negative) for few antigens, marked differences were seen in ALP expression (~50% of BMSCs vs ~25% of SASCs). But the differences in ALP expressions were not correlated with mineralization content.	126

^aLipogems are obtained by successive reduction and decantation of the clusters present in the liposyringes.

Table 3.
Representative List of Scaffolds Investigated for Regenerating Rotator Cuff Tissue

scaffold type	experiment	conclusion	ref
multi-layer tendon slices (COMTS)/BMSCs	<i>in vivo</i> (rat supraspinatus model, 6 weeks)	Both the tensile strength and the stiffness of COMTS/BMSCs were higher than the baseline values and those of COMTS alone at 6 weeks post implantation. H&E staining showed loose and fibrous capsule formation in non-augmented group, whereas the COMTS-only group, in addition, showed the presence of poorly organized fibroblast-like cells. In contrast, COMTS/BMSCs showed the presence of organized, homogeneous tissue with spindle-shaped fibroblasts aligned to the fiber axis, and a tide mark at the end of the fibrous tissue.	244
ovine forestomach ECM (OFM) scaffold	<i>in vitro</i> (cytocompatibility, immunogenicity) and <i>in vivo</i> (rat supraspinatus model, 12 weeks)	The hydrated scaffolds measuring 5 × 10 mm grafts were overlaid longitudinally along the tendon–bone insertion and incorporated by a suture. Higher fluorescence intensity (calcein AM), alamar blue assay, and low levels of dendritic cell expressions demonstrated excellent cytocompatibility of OFM with tenocytes. Despite similar mechanical properties, higher total histologic scores were observed in the augmented group, compared to the non-augmented group.	132
GraftJacket (GJ) vs SIS scaffolds/tenocytes	<i>in vitro</i> study (rat tenocytes, 7 days)	<i>In vitro</i> assay showed higher cell proliferation, TGF- β 1, FN, and PG content of healthy tenocytes on GJ scaffolds, compared to SIS, and lower than TCP for WST-1. Similar trend was also seen with glucocorticoid-treated seeded scaffolds, with SIS scaffolds showing high IL-6 contents but generally lower contents of TGF- β 1, FN, and PG.	135
acellular matrix (GJ) augmentation for rotator cuff repair	<i>in vivo</i> (clinical study, $n = 42$; mean age = 56 years; follow-up = 12–24 months)	Clinical outcome American Shoulder and Elbow Surgeons (ASES) scores demonstrated an improvement from 48.5 to 98.9 (SD = ± 4.2) in group 1 (augmented), and from 46.0 to 94.8 (SD = ± 14.2) in group 2 (non-augmented), with group 1 statistically better than group 2. Similar trend was also seen in Constant score, which showed increases from 41.0 to 91.9 (SD = ± 9.2) for group 1, and from 45.8 to 85.3 (SD = ± 11.0) for group 2. However, UCLA scores did not show such an increase, with similar end values. In addition, MRI showed a large number of intact cuffs in group 1 (17/20) (85%) compared to group 2 (6/15) (40%). Most common cause of adverse events was re-tears (3 for group 1, and 9 for group 2).	130
melt-spun PLA (~20 μ m) felts	<i>in vitro</i> (hydrolytic degradation in PBS) and <i>in vivo</i> (degradation in rabbits and tissue regeneration in dogs)	Hydrolytic degradation experiment on scaffolds measuring 8×40×2.5 mm indicated a statistical difference in the molecular weight from 8 weeks and beyond (12 and 16 weeks) ($P < 0.01$), but not at 6 weeks. Similar trend was seen in scaffolds measuring 8×25×2.5 mm implanted in rabbits <i>in vivo</i> , where statistical difference was seen in molecular weight at 12 and 16 weeks ($P < 0.01$), compared to initial molecular weight. Mechanical properties of the felts exposed to PBS retained its ultimate strength (6, 8, 12, and 16 weeks) and stiffness at all time points (except 8 weeks). In dogs, both strength and stiffness increased with implantation times.	245
silk microfiber/collagen fiber (or collagen sponge) scaffold	<i>in vitro</i> (cell culture, mechanical analysis) and <i>in vivo</i> (rabbit infraspinatus rotator cuff model, 12 weeks)	In collagen sponge/silk fiber (SCS) scaffolds, <i>in vitro</i> cell experiments showed random cell distribution with smaller cell lengths (low extension), whereas in 3-D collagen fiber/silk fiber (ACS) scaffolds, cells were aligned along the fiber axis and had larger cell lengths. These results corroborated well with <i>in vivo</i> experiments that showed higher cell numbers infiltrated in the ACS scaffold than in the SCS scaffold and histologic scoring demonstrating regeneration of tissue closely resembling the native tissue.	246
PCL microfibers/extracts from decellularized tendon ECM (tECM)/human ADSCs	<i>in vitro</i> , 2 weeks (differentiation, proliferation, gene expression, and matrix production)	The ADSCs were seeded on the scaffolds (and on TCP) and cultured for up to 2 weeks. At 7-day time point, DAPI staining showed high cell density in TCP when cultured with media containing tECM and FBS, which were further corroborated by MTS assay. The TCP-grown cells also showed up-regulation of Sca and TN-C expressions when cultured under tECM and FBS, with or without TGF- β 3. Further seeding in aligned PCL fibers showed increases in cell proliferation with the presence of FBS, but tenogenic differentiation increased significantly only in the presence of tECM.	247
woven P4HB mesh embedded in a collagen matrix/F2A peptide	<i>in vivo</i> (sheep infraspinatus rotator cuff model, 26 weeks)	The woven scaffolds embedded in collagen matrix coated with pre-determined amounts of F2A peptide (0, 1, 8, or 32 mg), after implantation for 8 and 26 weeks, were evaluated by histological, radiological, and biomechanical means. Histologic analyses showed subsiding moving scores in all groups, indicating absence of tendinopathy. In addition, new bone formation was observed with the addition of F2A, but not necessarily in a linear fashion; however, delamination considerably decreased and % tissue covered in implant site increased with increasing F2A amount.	172
woven PLA (X-repair device)	<i>in vivo</i> (canine infraspinatus intra-articular injury model, 12 weeks)	After causing excision and modeling intra-articular injury, woven scaffolds measuring 12×34×0.8 mm were affixed to the injury site. Non-augmented repairs led to larger tendon retractions (>2 cm), whereas augmented repairs led to smaller tendon retractions (<1 cm) after 12 weeks of healing.	166

scaffold type	experiment	conclusion	ref
PLGA nanofibers (3–14 μ m)	<i>in vivo</i> (rabbit rotator cuff model, 16 weeks)	After exposing the infraspinatus tendons, a defect size of 5×5 mm was created by resecting the infraspinatus tendons, and proximal scaffold was attached to the midsubstance of the tendon. H&E staining showed formation of bone (8 weeks) and cartilage (16 weeks) with cell alignment closely mimicking the native enthesis in scaffold augmented groups, whereas in the re-attachment group, even though bone formation was seen by 8 weeks, no tidemark formation was observed at even 16 weeks. In Safranin-O staining, the key difference was in the decrease in PG content at 16 weeks in the case of reattachment group.	248
polydioxanone (PDO) nanofibers seeded with healthy and diseased tendon cells	<i>in vitro</i> cell study (7-day study)	After cell seeding (0–24 h time points), the key difference was seen in the morphology between the cell types, with healthy cells more elongated than the diseased cells, due to the absence of CD106+, CD10+, and CD26+ cell fractions. Finally, between the scaffold types (PDO fibers and PDO discs), gene expression analyses showed low expressions of COL1A1 in nanofibrous samples and in scaffolds seeded with diseased cells, compared to their respective groups.	249
three layers of PLGA nanofibers (400–900 nm) + two layers of fibrin gels (to deliver ADSCs)	<i>in vivo</i> (rat infraspinatus model, 8 weeks)	All groups containing scaffolds showed a delayed response, compared to the suture-only group. However, cellularity scores, fibroblast cell numbers, collagen orientation, and insertion morphology were higher than normal and in the suture-only group. Addition of BMP-2 to the cellular group showed initial (28 days) lower trabecular bone volume, number, and spacing than cellular (no BMP-2) and acellular groups but normalizes toward a later time point (56 days).	250
PDO nanofibers/woven PDO microfibers bonded by a molten PCL film	<i>in vitro</i> (cell culture, cell viability and adhesion, mechanical evaluation) and <i>in vivo</i> (rat rotator cuff tendon model, 12 weeks)	Mechanical evaluation showed tensile strength, modulus, and strain at failure of composite scaffolds (woven + nanofibers) to be identical to those of woven fibers alone, but significantly higher than those of commercial rotator cuff grafts. Both cell proliferation and gene expression levels of tendon markers (COL1A1, COL3A1, fibrillin-2) were higher in scaffolds containing nanofibers compared to woven constructs.	251
PCL nanofibers seeded with autologous stem cells	<i>in vivo</i> (rat infraspinatus rotator cuff model, 8 weeks)	Cell tracking experiments showed stabilized implant structure (in tendon) at 8 weeks; however, attachment was disrupted in the bone. Biomechanical evaluation showed cells + scaffold group had higher stiffness and modulus values when seeded with juvenile and aged tendon cells, but not with adult tendon cells. A similar trend was also seen in maximum stress values with scaffolds seeded with aged tendon cells, but not with other tendon cells.	252
polyphosphazenes-coated PCL nanofibers seeded with rat MSCs	<i>in vivo</i> (rat supraspinatus rotator cuff model, 12 weeks)	After rotator cuff injury was induced, the torn tendons were repaired using modified Mason Allen switch or mechanically augmented with scaffolds (with or without seeded MSCs). With mechanical augmentation and MSCs seeding, a gradual transition in collagen orientation with low cellular density was observed, similar to that of native untorn tissue, whereas in the suture repair-only group, even at 12 weeks, a disorganized collagen matrix comprised of a higher density of rounded cells was found. In addition, an abrupt transition to bone with no cartilaginous material in between the bony site and tendon insertion was observed. In line with morphological analyses, a high COL1:COL3 ratio was observed in mid substance, similar to those in native untorn rotator cuff tissue. In suture-only group, however, a ratio of COL1:COL3 was found, indicative of possible scar tissue formation. Collagen orientation measured by birefringence analyses further revealed high birefringence values (4-fold higher than suture repair or mechanical augmentation groups) in MSC-seeded scaffold group, with values closely resembling that of native tissue.	253
layered PLCL and PGA sheets	<i>in vivo</i> (rabbit infraspinatus rotator cuff model, 24 weeks)	After resecting 10×5 mm at insertion of infraspinatus to bone, PGA scaffold measuring 10×5×1 mm was inserted into the distal region of the bony trough, and the proximal end was sutured to the infraspinatus region in the left shoulders. In right shoulders, using a similar technique, PLCL scaffolds were inserted. Histologic analyses showed PLCL sheets covered with scar-like tissue and presence of randomly oriented cells (fibroblasts in insertions and chondrocytes in tendon proper) and small diameter fibers at 4 weeks. In PGA group, scar tissue formation at 4 weeks and substitution of PGA fibers by tendon–scar tissue at 16 weeks in tendon insertion site. However, tendon–bone healing was observed with formation of continuous aligned collagen fiber to tendon axis, and fibrocartilage at 16 weeks.	254
open-cell PLA sheet (OPLA)/BMSCs	<i>in vitro</i> (cell tracking) and <i>in vivo</i> study (rabbit infraspinatus rotator cuff model, 6 weeks)	After 2 and 6 weeks of implantation, large numbers of viable cells were observed in OPLA/cell scaffolds, compared to none in OPLA scaffolds alone, although the macrophage numbers were similar in both groups at all measured time points. Similarly, at all time points, more immature fibroblasts were observed to be present in OPLA/MSC scaffolds. Finally, immunohistochemical staining showed production of large volumes of type-I collagen in OPLA/MSC scaffolds than OPLA alone, but similar type-II collagen contents in both groups.	255
PGA/PDO sheets vs collagen sponge (col-spo) seeded with tendon cells (from LHB)	<i>in vitro</i> (cell proliferation, gene analysis, mechanical, H&E staining, 14 days)	RT-PCR analysis of the tendon cells showed higher expression levels of COL1A1, decorin, and low levels of COL3A1 and Scx. The mechanical properties of the scaffolds showed 7 times higher tensile strengths in col-spo scaffold. <i>In vitro</i> assay (WST-1) showed similar cell numbers after 14 days of cell culture. By H&E staining, the study showed significant infiltration	255

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scaffold type	experiment	conclusion	ref
PGA sheets (PGA) vs fascia lata (FL) patch	clinical trial on humans with irreparable rotator cuff tears (62 patients)	of the cells through the col-spo scaffolds by 14 days, whereas at 2 days, cells were capable of infiltrating the upper layers only. Japanese Orthopaedic Association (JOA) shoulder rating scale was utilized to evaluate patients pre- and post-operatively at 12 months. JOA scale showed improvements from 54.9 ± 1.1 to 90.7 ± 1.0 points in PGA group. Similar improvements were also seen in FL group (52.6 ± 1.5 to 91.7 ± 1.2 points). One-year post surgery, MRI showed low re-tear rates in PGA group (13.3%), compared to 31.2% in FL group.	256