Growth Factors for Rotator Cuff Repair

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KEYWORDS

• Tendon biology • Cytokines • BMP • Growth factors
• Gene therapy • Repair scaffolds

Rotator cuff repair surgeries are one of the most common procedures performed by orthopedic surgeons, with over 250,000 performed annually in the United States alone. Despite its prevalence, there is concern regarding the ability of the rotator cuff to heal back to the insertion site on the humerus following repair. Clinical studies have shown radiographic failures at the repair site at 2 years in anywhere from 11% to 95% of patients, depending on the size and chronicity of the tear, presence of fatty infiltration, and the age and general health status of the patient.[1–6] Although patients with re-tears or failed healing may have pain relief, these studies show that they have inferior functional results when compared with patients with healed repairs.[2,3] An understanding of the histology and biology that occur during the healing process may lead to therapies that can improve the healing rate and improve the functional results of patients following repair.

Our understanding of tendon healing is largely based on animal studies because there is little histologic information on healing rotator cuff tendons in human beings. From this animal data, it is known that rotator cuff healing occurs in 3 stages: inflammation, repair, and remodeling (**Fig. 1**).[7] In the inflammatory stage, inflammatory cells migrate into the repair site guided by chemotactic factors followed by an influx of blood vessels and fibroblasts. In the repair phase, several growth factors are upregulated that induce cellular proliferation and matrix deposition. Finally, this tissue undergoes remodeling due to extracellular matrix turnover mediated by matrix metalloproteinases (MMPs).

At the conclusion of the healing process, a normal rotator cuff insertion site is not regenerated. Normally, the rotator cuff inserts into bone through 4 distinct transition zones: tendon, unmineralized fibrocartilage, mineralized fibrocartilage, and bone. After repair, the tendon heals to bone with an interposed layer of fibrovascular scar tissue that persists (**Fig. 2**A and B).[7–9] The mechanical properties of this fibrous tissue are weaker than the native insertion site and may render repairs prone to failure.

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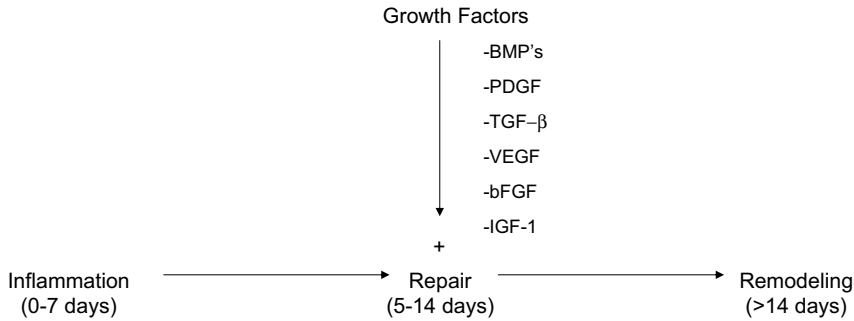

Fig. 1. The stages of rotator cuff healing involve inflammation, repair, and remodeling. Growth factors are expressed during the repair phase, because they promote cell proliferation and matrix production. This timeline must be kept in mind in growth factor therapies, because the addition of growth factors too early or late in the healing process may decrease their effectiveness.

In an effort to limit failures, researchers have focused on ways to minimize the formation of scar tissue at the interface, while at the same time promoting the regeneration of the fibrocartilaginous insertion zones. Initial studies have focused on improving the biomechanical strength of the repair through stronger sutures and by recreating the surface area of the footprint through double-row repairs or their equivalent. Even with these techniques, re-tears or failed healing still occur in up to 12% of patients.[4] Although improved biomechanics may modestly improve healing, it appears that biologic augmentation of the healing process is needed to further reduce failure rates. Biologic therapies that can limit the amount of scar tissue formation at the repair site, and help regenerate a normal fibrocartilaginous transition zone, may theoretically improve the strength of repairs.

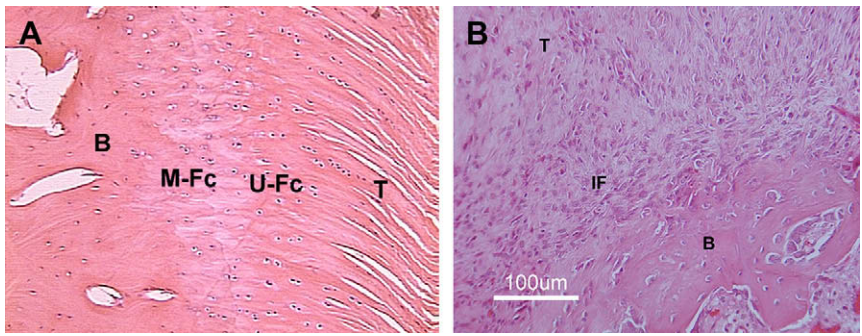

Fig. 2. (*A*) Histologic section of a normal supraspinatus tendon-insertion site in a rabbit, demonstrating the 4 zones of a direct insertion. T, tendon; U-Fc, unmineralized fibrocartilage; M-Fc, mineralized fibrocartilage; B, bone. (*B*) Histologic sections of the tendon-bone attachment site 4 wk after supraspinatus tendon repair in a rat. The resulting attachment site is characterized by a fibrovascular scar tissue interface (IF), without formation of an intermediate zone of fibrocartilage between tendon (T) and bone (B). (*Reprinted from* Rodeo SA. Biologic augmentation of rotator cuff tendon repair. J Shoulder Elbow Surg 2007;16(5S):191S–7S; with permission.)

Growth factors play an important role in cell chemotaxis, proliferation, matrix synthesis, and cell differentiation. Several growth factors are upregulated during the rotator cuff healing process. Basic fibroblast growth factor (bFGF), bone morphogenetic protein 12 (BMP-12), BMP-13, BMP-14, cartilage oligomeric matrix protein (COMP), connective tissue growth factor (CTGF), platelet-derived growth factor beta (PDGF-B), and transforming growth factor-beta 1 (TGF-β1) have all been shown to be upregulated during the normal healing process of a rat supraspinatus tendon.[10] Because these factors are present during the normal repair process of rotator cuff healing, the theory is that exogenous addition of these factors can further augment the healing process, much as BMP-2 and 7 have done for bone healing.

Several challenges exist in developing an effective biologic therapy to augment rotator cuff healing. First, the most effective growth factor or combination of growth factors must be determined. As research progresses, it is clear that a single factor therapy may not be sufficient. Rather, it is probable that several factors may be necessary, and the various possible combinations are numerous.

The second challenge is determining the optimum time for growth factor delivery. Growth factors are upregulated during the healing process in a temporal fashion, with most growth factors being upregulated 1 week following repair in rat models.[10–12] In the first week after injury/repair, the healing process is in the inflammatory phase. It is possible that this inflammatory response may override any anabolic agent that is added at this time. Therefore, timing of growth factor application is critical. This is supported by a study by Chan and colleagues[13] in which they found that addition of PDGF into a rat patellar tendon defect at 3 days had no effect on the biomechanical strength of the repair, whereas PDGF injection on day 7 improved peak loads-to-failure. Therefore, any growth factor added at the time of surgery needs to be incorporated into a sustained-release drug delivery vehicle that ensures that the factor is present during the regenerative phase of healing.

The final challenge involves developing a delivery vehicle for the growth factor. Many rotator cuff repair surgeries are now performed arthroscopically, so the delivery vehicle must be amenable to placement through cannulas and the growth factor must not be eluted in the fluid-filled arthroscopic environment. These technical considerations make gels, pastes, cements, and glues less desirable than scaffolds or patches.

In this review, the most recent research into the ability of growth factors to augment rotator cuff healing is discussed. Because healing depends on tendon-to-bone healing at the footprint, as well as tendon-to-tendon healing for side-to-side repairs, investigations that examine both processes are discussed. This is followed by a brief review on novel advances for the delivery of growth factors to the repair site. At the conclusion of the review, the reader should have an understanding of the various growth factors that have been highlighted as being potentially clinically useful, an appreciation for the recent research into delivery modalities, and the challenges of growth factor therapy for augmentation of rotator cuff repair.

STUDIES ON THE APPLICATION OF GROWTH FACTORS TO IMPROVE HEALING

In animal models, growth factors are effective in increasing the cellularity and overall tissue volume at the repair site. These findings usually result in increased failure loads on biomechanical testing; however, these failure loads become less significant when they are normalized to the volume or cross-sectional area of the repaired tissue. This implies that growth factors are able to improve the strength of the repair by promoting the formation of more scar tissue (ie, the structural properties are improved but the material properties are not improved). Excessive scar tissue at the healing attachment

site may predispose patients to impingement postoperatively. The ultimate outcome of the repair depends on both pullout strength and stiffness. Stiffness and creep may be more important parameters. Ideally, biologic therapies are able to induce tissue formation with material properties close to that of normal tissue.

Osteoinductive Proteins

Secure healing between tendon and bone requires bone ingrowth into the fibrovascular scar tissue and outer tendon. Therefore, factors that induce bone formation may theoretically improve the strength of the repair. Several studies have used this strategy to improve tendon healing in a bone tunnel in animal models, analogous to an anterior cruciate ligament (ACL) repair. However, there are few studies on improving rotator cuff repairs with osteoinductive factors. Rodeo and colleagues[14] studied the effects of an osteoinductive bone protein extract derived from bovine cortical bone (Sulzer Biologics, Wheat Ridge, CO) in a sheep model. This extract contains BMPs 2 through 7, TGF-β1, TGF-β2, TGF-β3, and FGF. The experimental group received 1.0 mg of the bone protein extract on a type-I collagen sponge, which was placed between the infraspinatus and the bone before repair and animals were sacrificed at 6 weeks and 12 weeks. Based on magnetic resonance imaging, repairs that received the bone-protein extract had a greater volume of bone and soft tissue at the repair site at both time points when compared with controls. Histologic examination showed significantly more fibrocartilage between the tendon and the bone in the experimental group. The imaging and histology results correlated with greater failure loads at both 6 and 12 weeks in the treated group. However, when the failure loads were normalized by tissue volume, there were no differences between groups. This suggests that growth factor treatment resulted in the formation of poor-quality scar tissue rather than true tissue regeneration.

Bone Morphogenetic Proteins-12 and -13 (BMP-12 and -13)

BMP-12 (also known as growth and differentiation factor 7) and BMP-13 (growth and differentiation factor 6) are both expressed at the embryonic development sites that form tendons and their insertions.[15] These molecules are distinct from the osteoinductive BMPs (BMP-2,-4,-7) and induce formation of tendon and fibrocartilage. Studies have reported that administration of recombinant human BMP-12 (rhBMP-12) and rhBMP-13 leads to induction of neo-tendon/ligament formation in rats and improved healing of tendon laceration.[15–17] A study conducted in conjunction with Wyeth Research, Inc., investigated the effects of rhBMP-12 (Wyeth Research, Cambridge, MA) on rotator cuff tendon-bone healing in a sheep model.[18] In this study, 4 treatment groups were evaluated: rhBMP-12 in injectable hyaluronan paste, rhBMP-12 in hyaluronan sponge, rhBMP-12 in absorbable type-I collagen sponge, and rhBMP-12 type-I/III collagen sponge. These were compared with a control group that underwent detachment and repair of the infraspinatus. At 8 weeks, specimens treated with rhBMP-12 in collagen sponges were 2.7 times stronger than untreated specimens, whereas those treated with rhBMP-12 in hyaluronan sponges were 2.1 times stronger than controls. Interestingly, specimens treated with rhBMP-12 in hyaluronan paste were similar to untreated controls, again demonstrating the importance of the delivery vehicle in growth factor therapy. Histologic evaluation found reestablishment of collagen fiber continuity between the bone and the fibrovascular interface scar tissue, with increased glycosaminoglycan content in the rhBMP-12-treated specimens. These results suggest that rhBMP-12 may be useful in improving rotator cuff repair healing.

Platelet-Derived Growth Factor

PDGF-BB has been found to act as a mitogen and chemotactic cytokine that can potentially enhance ligament and tendon healing. In a rat model of knee medial ligament (MCL) healing after transection, it was found that treatment with PDGF alone when compared with a combination of growth factors improved the structural properties of the femoral-MCL-tibial complexes.[19] In a rabbit knee medial collateral ligament rupture model, the application of PDGF-BB delivered in fibrin sealant significantly improved the ultimate load, energy absorbed to failure, and ultimate elongation values of the femur–MCL–tibia complex when compared with the control group.[20] In a rat patellar-tendon defect model, there was an increased proliferative response when PDGF-BB was supplemented on day 3 after surgery by way of syringe injection, whereas supplementation on day 7 improved peak load and pyridinoline content after administration of the highest dosage of PDGF.[13] In a rat model of rotator cuff repair, delivery of cells expressing PDGF-BB with a polyglycolic acid (PGA) scaffold showed restoration of normal crimp patterning and collagen bundle alignment compared with suture repair only.[21] A study conducted in the authors' laboratory evaluated the ability of PDGF-BB on a collagen scaffold to improve rotator cuff healing in a rat. Increased cellular proliferation and angiogenesis were found in a dose-dependant fashion at 5 days; however, this did not correlate with improved healing at 28 days based on histology or biomechanical testing.[22] These studies demonstrate that improved healing with PDGF is dependent on the dosage, timing, and delivery vehicle used.

There are currently several commercially available systems to create a ''platelet-rich plasma'' or ''platelet gel'' from autologous blood. These systems involve spinning autologous blood in a centrifuge to form a dense, suturable fibrin matrix that can be easily placed directly at the tendon repair site. One technical problem with these systems is that many use human or bovine thrombin to form the platelet-rich plasma. Excess thrombin causes premature platelet activation and degranulation, causing immediate release of the platelet-derived cytokines. Newer systems have omitted the use of thrombin to prevent this phenomenon during processing. Currently, there are no clinical studies on the efficacy of this treatment though theoretically it holds promise.

Transforming Growth Factor-β

During wound healing, TGF-β is released from degranulating platelets and secreted by all the major cell types participating in the healing process, including lymphocytes, macrophages, endothelial cells, smooth muscle cells, epithelial cells, and fibroblasts.[23] Scar tissue formation has been closely associated with the presence of the 3 TGF-β isoforms (TBF-β1, 2, and 3). Although adult wounds heal with an abundance of scar tissue, which is correlated with increased expression of TGF- β1, fetal wounds heal without scar and without expression of TBF-β1. Therefore, inhibition of TGF-β1 or exogenous application of TGF-β3 may reduce scar tissue formation in the interface. TBF-β3 is expressed during fetal tendon development. In a study on a rat rotator cuff repair model, Kim and colleagues[24] found that exogenous application of TGF-β3 resulted in improved mechanical properties when compared to specimens treated with TGF-β1. Conversely, application of TGF-β1 coupled with suppression of TGF-β2 and -3 led to mechanically inferior tissue despite increased cross-sectional area. This suggests that although TGF-β1 results in the exuberant production of scar tissue at the repair site, this tissue is mechanically weaker than normal tissue. The ultimate goal in developing strategies to improve rotator cuff healing is to limit the amount of scar formation, while maximizing the strength of the repair.

Vascular Endothelial Growth Factor

It is well established that the rotator cuff tendon is hypovascular in the area adjacent to the distal insertion site.[1] Therefore, it seems reasonable that increased vascularity could improve rotator cuff healing. VEGF is known to have a potent angiogenic effect and is expressed in high concentration in healing flexor tendons 7 to 10 days following repair, with a return to normal by 14 days.[10] No studies have directly evaluated the role of these molecules in rotator cuff repair. However, Zhang and colleagues[25] injected VEGF into repaired Achilles tendons in a rat model and found improved tensile strength early in the course of healing. In contrast, a recent study on the ability of VEGF on graft healing in a sheep ACL reconstruction model showed no benefit in VEGF therapy over controls.[26] In this study, the grafts were soaked in VEGF in the experimental group, whereas the control group grafts were soaked in phosphate buffered saline. Although there was increased vascularity in the VEGF-treated group, the stiffness of the femur–graft–tibia complex in the VEGF-treated group was significantly lower than in controls. Although only a single concentration of VEGF solution was used, and the animals were evaluated at only 1 time point (12 weeks), these preliminary data suggest that excessive vascularity may have detrimental effects on the healing ACL graft. It is unclear if these findings can be extrapolated to rotator cuff healing.

Basic Fibroblast Growth Factor

bFGF causes fibroblasts to produce collagenase and stimulates proliferation of capillary endothelial cells, both of which are necessary for angiogenesis. It also helps to initiate the formation of granulation tissue. In vitro work has shown bFGF results in cell proliferation and collagen production in cultured flexor-tendon tenocytes.[27,28]

Recent in vivo work also appears encouraging, though there are no studies to date that have evaluated bFGF in a rotator cuff repair model. Chan and colleagues[29] injected bFGF in various doses into rat patella tendons 3 days after a window defect was created. At 7 days, there was a dose-dependent increase in the number of proliferating cells and the level of expression of type-III collagen. However, these results were not seen at 14 days, nor were there any differences in the ultimate stress and the pyridinoline content of the healing tendons. Tang and colleagues[30] used a digital flexor-tendon repair model in chickens to evaluate the efficacy of injecting bFGF in an adeno-associated viral vector into the lacerated tendon ends before repair. They found that tendons treated with this vector had increased ultimate loads-to-failure when compared with those treated with a sham vector, or no vector at all, at 2, 4, and 8 weeks. Exogenous bFGF loaded onto a monofilament nylon suture has also been shown to result in more cellularity and increased failure loads at 3 weeks in another flexor-tendon repair model.[31]

Insulin-Like Growth Factor-1

IGF-1 has been shown to have anabolic effects on healing tendons by stimulating protein synthesis, increasing cell proliferation, collagen synthesis, and decreasing swelling. In vitro studies have shown that the addition of IGF-1 to tenocytes in culture induces matrix synthesis, but did not affect matrix turnover.[28] Kurtz and colleagues[32] applied exogenous IGF-1 to repaired rat Achilles tendons and found that it stimulated the synthesis of DNA, collagen, and proteoglycans and that this resulted in reduced time to functional recovery. Dines et al. studied the ability of rat tendon fibroblasts transduced with a retroviral vector containing IGF-1.[33] These cells were then seeded onto a bioabsorbable polymer scaffold that was made of nonwoven, PGA. The

scaffold was then tested in a rat rotator cuff model. At 6 weeks, specimens treated with the IGF-1 seeded scaffold exhibited better histology scores and a higher ultimate load-to-failure than those with the scaffold alone. This study introduced a novel manner by which to deliver growth factors to the healing repair site, and its results are encouraging and warrant further investigation in larger animal models.

DEVELOPMENTAL BIOLOGY AS A PARADIGM FOR TENDON REGENERATION

Work in developmental biology laboratories have identified several molecules that are thought to play a role in tendon and tendon-bone development during embryogenesis. The theory is that an understanding of the mechanisms by which tendons and tendon-bone interfaces are formed in the fetus will one day lead to therapies that can induce regeneration of normal tissue as opposed to the fibrosis seen in adults. Scleraxis is a transcription factor that is upregulated in tissues that develop into tendons, leading researchers to postulate that it plays a role in driving tenocyte differentiation.[34] Shukanami and colleagues[35] linked scleraxis expression with another tenocyte marker, a transmembrane glycoprotein named tenomodulin. The role these proteins play in the formation of tendons is still unclear, but they have been shown to result in tenocyte proliferation. There have been no in vivo studies investigating their ability to improve rotator cuff healing.

Initial formation of tendons occurs independently with respect to muscle, but later development depends on signals from the muscle to drive tendon development and maturation. FGF-4 is secreted from the muscle of developing chick embryos. Its presence results in upregulation of scleraxis and another tendon marker, tenascin.[36] This implies that FGF-4 may be responsible for proliferation of tenocytes and maturation of tendons during development. Another protein necessary for tendon development is myostatin (GDF-8). Mendias and colleagues[37] showed that the tendons of myostatin knockout mice were smaller, more brittle, had less cellularity, and had a decrease in the expression of type-I collagen. Conversely, treatment of tendon fibroblasts with myostatin activated tenocyte proliferation pathways and increased the production of type-I collagen. Although the field of tendon development and its translational application to the augmentation of rotator cuff repairs is in its infancy, the possible therapies this research can lead to is exciting.

GROWTH FACTOR DELIVERY METHODS

Perhaps the most challenging aspect of growth factor therapy for the augmentation of rotator cuff repairs is determining a way to deliver the factor to the healing site. As discussed, once the proper combination of growth factors has been determined, as well as their optimal time for delivery, they then need to be delivered to the tendon-bone healing site in a fluid-filled arthroscopic environment without interfering with healing. Gene therapy approaches and tissue regenerative scaffolds are currently being investigated.

Gene Therapy

Gene therapy was first developed to treat inherited genetic defects by replacing a defective copy of the gene with a normal one. In orthopedics, however, attention has been turned to this technique as a biologic sustained release, local growth factor delivery vehicle. There are 2 main strategies for growth factor delivery with gene therapy, ''ex vivo'' and ''in vivo'' (**Fig. 3**).[38] The ex vivo technique involves transferring the gene that codes for the growth factor of interest into carrier cells in vitro. These cells then overexpress the growth factor for which the gene codes for and releases it into the

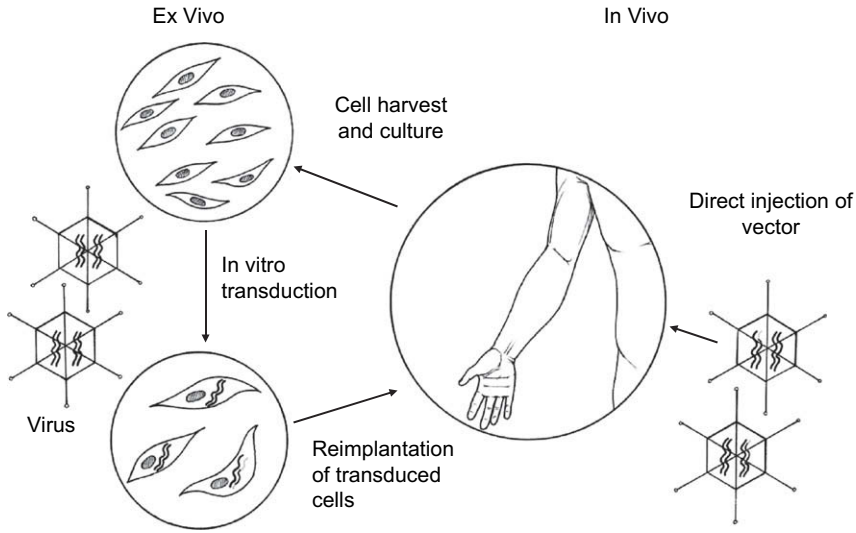

Fig. 3. The 2 basic gene therapy strategies: in vivo and ex vivo. The in vivo strategy involves administering the vector containing the gene of interest directly to the patient. The ex vivo method involves harvesting cells, transducing them with the vector containing the gene in vitro, then re-administering the cells into the repair site. (*Reprinted from* Musgrave DS, Fu FH, Huard J. Gene therapy and tissue engineering in orthopaedic surgery. J Am Acad Orthop Surg 2002;10:6–15; with permission.)

local environment. The transduced stem cells are then added to the repair site where they release the growth factor for an extended period of time. The second, less common, method involves delivering the gene of interest directly into the local cells of the healing tissue. This involves exposing the host tissue to the vector containing the gene of interest. This technique is less attractive because the vector is usually a virus, and there is a risk of contaminating the surrounding tissue and the surgeon.

Tissue Engineering Scaffolds and Coated Sutures

Scaffolds are 3-dimensional structures that promote regeneration of the surrounding tissue. The scaffold by itself may guide new tissue formation by its 3-dimensional architecture. It may also be seeded with either cells, growth factors, or both. Scaffolds can be made of naturally derived polymers, such as collagen and hyaluronic acid, synthetic polymers, such as polyL-lactic acid (PLLA), PGA, polyDL-lactic-co-glycolic acid (PLGA), polyvinyl alcohol (PVA), or injectable polymers that cross-link in situ, such as alginate and polyethylene oxide (PEO). In the simplest design, the various scaffolds can be soaked in a solution containing the growth factor such that the factor wicks onto it. This scaffold can then be added to the repair site where the growth factor is eluted into the local environment. This technique has been outlined by Dines and colleagues,[39] in which a Vicryl (polyglactin 910, Ethicon, Somerville, NJ) was coated with rhGDF-5. They showed that a consistent amount of growth factor is released from the sutures after passage through soft tissue.

More complex strategies have also been outlined in which hydrogels are embedded with microspheres that contain growth factors. The microspheres then are able to release the factor at a controlled rate. This technique offers the option to load the scaffold with more than 1 growth factor. Furthermore, the microspheres can be engineered to release different growth factors at different rates, so that the factors can be released

in a temporal fashion. Although research in this field has progressed substantially, these technologies are still far from being clinically useful.

SUMMARY

The 4 fibrocartilaginous transition zones of the rotator cuff insertion site are not recreated following surgical repair. Instead, a layer of scar tissue is formed between the tendon and the bone, which renders repairs prone to failure. Growth factors are a group of cytokines that induce mitosis, extracellular matrix production, neovascularization, cell maturation, and differentiation. Research has focused on their ability to augment rotator cuff repairs. Studies have shown that several factors are capable of increasing the strength of repairs in animal models. However, this appears to be accomplished through the production of more scar tissue, as opposed to regeneration of native tissue. It is becoming clear that multiple factors may be needed to regenerate the native tendon-bone insertion site. The optimal timing and vehicle for growth factor deliver have remained elusive. Gene therapy and tissue scaffolds provide promising options for the future, but the engineering still needs to be optimized for clinical use. Growth factor therapy for rotator cuff repairs remains a promising therapeutic for the future; however, much work needs to be done to optimize its effectiveness.

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