Biologic and Tissue Engineering Strategies for Tendon Repair

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Abstract

This review summarizes recent developments in biologic treatments—including growth factors, platelet-rich plasma (PRP), stem cells, and cell-seeded scaffolds—for tendon repair. Growth and differentiation factor-5 (GDF-5), insulin-related growth factor-1 (IGF-1), and basic fibroblast growth factor (bFGF) all improved extracellular matrix (ECM) production and tensile strength of treated tendons; however, no clinical trials were done on GDF-5. Platelet-derived growth factor-BB (PDGF-BB) improved proliferation and ECM production, but did not consistently improve mechanical properties. The literature was mixed on the efficacy of PRP for the treatment of chronic and acute tendinopathies. However, PRP did cause any complications, and its benefits may be enhanced once an ideal, standardized composition is developed. Therefore, PRP may be a valid treatment, especially once nonsurgical management options have failed. Mesenchymal stem cells (MSCs) significantly and substantially improved the quality of tendon repairs and demonstrated the ability to regenerate an enthesis. Adipose-derived stem cells (ADSCs) have similar effects and are easier to harvest. The peritendium may also regenerate the tendon–bone attachment. Tenocytes, meanwhile, may be ideal for midsubstance tendon repairs. Cell-seeded scaffolds—especially ECM-derived scaffolds—were demonstrated to improve ECM

production, enhancing the healing abilities of tenocytes or stem cells while providing early mechanical support to healing tendons.

Each of these treatments demonstrated enhanced healing compared to common surgical techniques; moreover, patient outcomes may be enhanced by combining these treatments.

Lay Summary

Tendon injuries are very prevalent and can be debilitating. Tendon heals poorly, and the scar tissue that forms is weak and susceptible to reinjury. A major focus of orthopedic research is regenerative medicine, encouraging the formation of healthy tendon rather than mechanically inferior scar tissue. This review summarizes the recent scientific literature on biologic treatments for tendon repair, such as growth factors, platelet-rich plasma, and stem cells. The purpose of which is to show which treatments are promising candidates for clinical use and research, helping to guide physicians and to lay out a path for future research.

Keywords Tendon · Biologics · Growth factors · Platelet-rich plasma · Stem cells · Scaffolds

Introduction

Tendinopathy is among the most common injuries in athletes and the general population, affecting over 50 % of runners and up to 80 % of runners. Chronic, nonhealing tendon injuries frequently require surgical treatment, yet despite recent advancements in orthopedic surgery, many common tendon repair techniques yield less than optimal results [1–3]. Healed tendons tend to form scar tissue with different mechanical properties than healthy tendon

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tissue. This may be due to the variance in collagen types within the scar tissue compared with healthy tendon. Collagen type I (Col-I) predominates in health tissue, whereas collagen type III (Col-III) is more abundant after tendon repair, resulting in elastic, loosely organized fibrils [4–6]. These healed tendons, therefore, are more prone to reinjury. Moreover, many common techniques to repair tendon tears at the tendon–bone interface, such as the use of suture anchors, cannot regenerate the enthesis. The tendon and bone are thus only held together primarily by sutures, resulting in a high incidence of rerupture after tendon reattachment surgeries [7, 8]. Therefore, alternative surgical procedures may be required to ensure proper tendon healing.

Biologic augmentation is a promising strategy to enhance tendon repair and regeneration. Biologics refers to cell-based products and therapies that can promote cellular regeneration and differentiation. These materials may limit the formation of scar tissue with undesirable mechanical properties and, potentially, can aid in the regeneration of the tendon–bone interface. This article will focus on three developments in biologics with implications in tendon healing: growth factor application, platelet-rich plasma (PRP), and stem cell therapy.

PRP is blood plasma isolated via centrifuges and with platelet concentrations substantially higher than whole blood [9]. Platelets, when activated, secrete growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF- β), and fibroblast growth factor (FGF), which may promote healing, shorten the duration of recovery from surgery, and impede the formation of scar tissue [10]. Moreover, as PRP is derived from a patient's own blood, its use is associated with few complications [10]. For this reason, PRP administration has become an increasingly popular treatment for tendon injuries. Growth factors and mitogens such as those found in PRP can also be coated on scaffolds or sutures or applied directly to an injury site.

Stem cells can differentiate to replace damaged cells and tissues and have been thoroughly investigated as augments for tendon repair surgery. Stem cell therapies are particularly promising to promote the regeneration of the enthesis [11], thereby providing a more durable tendon–bone connection than suture anchors. Mesenchymal stem cells (MSCs) are the cell type most commonly investigated to promote tendon repair, although stem cells from a variety of sources have been considered. Stem cells often are delivered to an injury site through a scaffold or injection. A scaffold fills a defect or gap in a tendon, taking on much of the tendon's mechanical load until the tissue heals and the scaffolds degrades [12]. These scaffolds may also mimic the environment of uninjured tendon, guiding the differentiation of seeded cells [13].

Growth Factors

TGF- β Superfamily

Growth and differentiation factor 5 (GDF-5, also known as bone morphogenic protein-14) has been well studied in animal models for its role in tendon repair. In vitro studies have shown that GDF-5 may upregulate the expression of aggrecan, collagen types I and III (Col-I, Col-III), scleraxis, tenomodulin, and metalloproteinase 9 (MMP-9, an enzyme which promotes tenocyte infiltration) in mouse and rat tenocytes (Table 1) [14, 15]. Furthermore, GDF-5 augmentation increased proliferation and ECM production of tenocytes [15] and rat adipose-derived stem cells at a concentration of 100 ng/kg [16]. GDF-5 supplementation of human MSCs did not affect cell proliferation, but did increase total collagen synthesis, scleraxis and tenascin-C expression, and Col-I/III ratios at 100 ng/kg [17].

When compared with saline injections, 10 μ g GDF-5 injections downregulated proinflammatory genes, improved collagen organization, and promoted aggrecan, MMP-9, and fibromodulin expression in mouse Achilles tenotomy suture repairs [14]. GDF-5-coated sutures (at dosages of 24, 55, 556, and 1.0 μ g/cm) also increased stiffness, tensile strength, and cross-sectional area in lacerated rat Achilles tendons [18, 19]. GDF-5-coated sutures at 55 ng/cm improved collagen organization and maximal load of severed rabbit flexor tendons after 3 weeks (11.6 ± 3.5 N vs. 8.6 ± 3.0 for controls without GDF-5), although these values were not significant after 6 weeks [20]. GDF-5 gene therapy using an adenovirus vector was also shown to promote healing, lessening visible gapping and improving tensile strength by 70 % compared with sham virus controls in a rat Achilles model [21].

Although GDF-5 provides substantial healing benefits, other GDFs may also be effective. Aspenberg et al. found that the delivery of 10 μ g GDF-5 or 1 μ g GDF-6 (also known as bone morphogenic protein-13) via Col-I sponges enhanced tensile strength compared with controls receiving unseeded sponges in a rat model of Achilles repair [22], and ectopically implanted GDF-5, GDF-6, and GDF-7 (also known as bone morphogenic protein-12) at concentrations between 5 and 25 μ g were all found to induce neotendon formation in rats [23].

GDF-7 may have implications in stem cell preparations. GDF-7 at 10 ng/mL stimulated the expression of scleraxis and tenomodulin in vitro in rat MSCs. When Col-I scaffolds seeded with these GDF-7-primed MSCs were implanted in half-width defects of rat calcaneal tendons in vivo, the experimental tendons demonstrated enhanced matrix and cell organization compared with untreated MSCs [24]. Seeherman et al. found that Col-I sponges treated with rhGDF-7 (also known as BMP-12) more than doubled load-to-failure in an ovine model of rotator cuff repair when compared with

Table 1 Evidence of growth factor efficacy for tendon repair

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
GDF-5	In vitro followed by in vivo	In vitro tenocyte augmentation with GDF-5 followed by in vivo midsubstance tenotomy and suture repair with or without 10 µg GDF-5 injections	Achilles	Mice	Increased expression of aggrecan, Col.I, Col.III, scleraxis, tenomodulin, and MMP-9	Hogan et al. [14]
GDF-5	In vitro	Tenocytes augmented with varying GDF-5 concentrations	Achilles	Rat	Increased tenocyte proliferation and ECM production. Increased expression of aggrecan, Col.I, Col.III, scleraxis, tenomodulin, and MMP-9	Keller et al. [15]
GDF-5	In vitro	ADMSCs augmented with varying GDF-5 concentrations	–	Rat	Increased proliferation and ECM production of ADSCs	Park et al. [16]
GDF-5	In vitro	MSCs augmented with varying GDF-5 concentrations	–	Human	No increase in MSC proliferation Increase in collagen synthesis, scleraxis and tenascin-C expression	Tan et al. [17]
GDF-5	In vivo	Tendon insertion point resection and repair with dip-coated GDF-5 sutures	Rotator cuff	Rat	Increased stiffness, tensile strength, and cross-sectional area of lacerated tendons	Dines et al. [18]
GDF-5	In vivo	Tendon transection and repair with GDF-5 coated sutures	Achilles	Rat	Increased stiffness, tensile strength, and cross-sectional area of lacerated tendons	Rickert et al. [19]
GDF-5	In vivo	Tendon transection and repair with GDF-5 coated sutures	Zone II flexor tendon	Rabbit	Maximal load and collagen histology scores were higher for GDF-5 group than controls	Henn et al. [20]
GDF-5	In vivo	Tenotomy repair with GDF-5 gene therapy	Achilles	Rat	70 % greater tensile strength than controls, elevated neotendons, no inflammatory response, ectopic bone, or cartilage formation	Bolt et al. [21]
GDF-5 and GDF-6	In vivo	Tendon transection repair with GDF-5-soaked sponges	Achilles	Rat	GDF-5 at 10 µg and GDF-6 at 1 µg enhanced tensile strength compared with controls	Aspenberg et al. [22]
GDF-5, 6, and 7	In vivo	Quadriceps ectopic implants of GDF-5, GDF-6, and GDF-7	–	Rat	Each GDF 5, 6, and 7 implant induced formation of organized Col.I resembling neotendon	Wolfman et al. [23]
GDF-7	In vitro and in vivo	Rat bone marrow-derived MSCs were cultured in monolayer and treated with GDF-7. These cells were then implanted in half-width defects of the calcaneal tendon	Calcaneal	Rat	Administration of GDF-7 increased expression of scleraxis, tenomodulin, Col.I, and tenascin-c. When implanted in vivo, cells treated with GDF-7 increased cell number, matrix deposition, and alignment relative to untreated MSCs	Lee et al. [24]
GDF-7	In vivo		Infraspinatus	Ovine		Seeherman et al. [25]

Table 1 (continued)

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
GDF-7 and BMP-13	In vivo	Augmentation of double-row reattachment of the infraspinatus tendon with rhGDF-7 soaked Col.I/Col.III sponges Midsubstance tendon transections were left to heal unaided, or healing was enhanced by GDF-7 or BMP-13 injections	Achilles	Rat	Tensile strength in rhGDF-7 collagen sponges was 2.1 times higher than in tendons augmented with untreated sponges GDF-7 and BMP-13 both increased tissue volume and tenocyte infiltration. The rate of tendon repair as measured by tendon width over time was enhanced in augmented rats	Jelinsky et al. [26]
BMP-2 and BMP-7	In vitro	Tenocytes from human rotator cuff were isolated and cultured with BMP-2 or 7	Rotator cuff	Human	BMP-2 increased Col.I production, but not collagen expression. BMP-7 increased cell activity and Col.I production and expression	Pauly et al. [27]
BMP-2	In vivo	Flexor digitorum communis tendons were injected with BMP-2 to induce calcification, followed by tendon resection and bone-tunnel reattachment surgery after 4 weeks	Flexor digitorum communis	Rabbit	The procedure produced an enthesis-like structure at the reattachment site	Hashimoto et al. [28]
BMP-2	In vivo	Patellar tendon transection at insertion point followed by suture anchor repair utilizing fibrin glue or fibrin glue enhanced with BMP-2	Patellar	Rabbit	BMP-2 augmentation enhanced bone growth at the tendon-bone interface, resulting in a significant increase in failure loads of the repaired tendons	Kim et al. [29]
BMP-2	In vivo	Flexor digitorum profundus detachment and repair into bone tunnels	Flexor digitorum profundus	Canine	No significant differences in ultimate load or strain between BMP-2 and untreated tendons	Thomopoulos et al. [30]
TFG-β1 IGF-1 GDF-5	In vitro	Tendon stem cell culture	–	Rat	IGF-1 at 10 and 100 ng/mL maintained multipotency in tendon stem cells GDF-5 increased Col.I expression at 10 and 100 ng/mL after 28 days TFG-β1 upregulated Col.II expression at 1, 10, and 100 ng/mL	Holladay et al. [31]
TFG-β1	In vitro	Tenocytes cultured in Col.I hydrogels exposed to varying TGF-β1 concentrations	Flexor	Mice	Increased expression of Col.V, Col.XII, and scleraxis. No effect on Col.I synthesis	Farhat et al. [32]
TFG-β1	In vitro	Cultured flexor tendon endotenon, epitenon, and sheath cells were exposed to 2 ng/mL TGF-β1	Flexor	Rabbit	Decreased expression of Col.I in endotenon cells; increased expression of Col.III in all three cell types	Klass et al. [33]
TFG-β1	In vivo	Achilles transection repair by Tsuge method with TGF-β1 injection	Achilles	Rat		Kashiwagi et al. [34]

Table 1 (continued)

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
TGF- β 1	In vivo	Patellar tendon transection repair with TGF- β 1 injection	Patellar	Rabbit	Increased failure load and stiffness; dose-dependant increase in procollagen I and III expression	Anaguchi et al. [35]
IGF-1	In vitro	Equine tendon and bone marrow derived cells were cultured in monolayer with or without IGF-1	Superficial digital flexor	Equine	Improved tangent modulus and increased failure loads	Durgam et al. [36]
IGF-1 and FGF-2	In vitro	Tendon cells were expanded in monolayer with or without FGF-2 augmentation. Other tendon cells were cultured in an acellular pulverized tendon matrix with or without IGF-1	Superficial digital flexor	Equine	IGF-1 enhanced cell number and collagen and GAG synthesis	Durgam et al. [37]
IGF-1	In vitro	Superficial digital flexor tendon explants were cultured with or without IGF-1	Superficial digital flexor	Equine	FGF-2 significantly increased ECM and Col.III synthesis. IGF-1 increased and Col.I and Col.III expression	Dahlgren et al. [38]
rhIGF-1	Clinical study	Healthy patients were administered two 1 mg IGF-1 injections in one patellar tendon	Patellar	Human	IGF-1 increased DNA content by 31 %, GAG content by 29 %, and collagen synthesis by 72 % relative to untreated controls	Hansen et al. [39]
IGF-1	Clinical study	Healthy patients were administered one 1 mg IGF-1 injections in one patellar tendon	Patellar	Human	Collagen fractional synthesis rate increased by 25 % in IGF-1 tendons relative to saline-treated tendons	Nielsen et al. [40]
TGF- β 1 and IGF-1	In vivo	TGF- β 1 and IGF-1 were administered to patellar tendon defects via a fibrin sealant	Patellar	Rabbit	Collagen fractional synthesis rate increased by 100 % in IGF-1 tendons relative to saline-treated tendons	Lyrras et al. [41]
IGF-1	In vivo	Racehorses with superficial digital flexor tendonitis were administered 4–5 intralesional IGF-1 injections	Superficial digital flexor	Equine	Increased ultimate failure load, stress, and stiffness	Witte et al. [42]
IGF-1	In vivo	Collagenase-induced lesions of equine flexor digitorum superficialis tendon were treated with ten 2 μ g rhIGF-1 injections	Superficial digital flexor	Equine	Treatments reduced echolucency in 23 of 26 subjects, and 63 % were able to return to racing	Dahlgren et al. [43]
IGF-1	In vivo	MSCs and MSCs cultured with IGF-1 were used to treat SDF	Superficial digitorum flexor	Equine	IGF-1 significantly reduced lesion size at all timepoints over the 8-week study but did not affect ultimate failure load, GAG content, or Col-I:Col-III ratios	Schnabel et al. [44]
GH	Clinical study		Patellar	Human	MSC + IGF-1 improved histological scores but did not affect expression of Col-I or Col-III. There were no significant differences between the MSC and MSC + IGF-1 groups	Doessing et al. [45]

Table 1 (continued)

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
GH	Clinical study	Nine male patients with acromegaly and nine with GH deficiency underwent tissue biopsy	Patellar	Human	IGF-1 levels in patellar tendons of acromegalic patients were 2.9-fold higher and Col-I expression was 1.7-fold higher than in GH-deficient patients GH administration increased tendon Col-I expression 3.9-fold	Doessing et al. [46]
GH	In vivo	Ten healthy male patients were administered subcutaneous GH injections of 33.3 µg/kg/d+50 µg/kg/d for 14 days	Achilles	Rat	GH treatments had no effect on ultimate failure load or stiffness	Andersson et al. [47]
bFGF	In vitro	Rat Achilles tendons were transected and treated with GH injections twice daily for ten days	Flexor	Canine	2-fold increase in tenocyte proliferation relative to controls	Thomopoulos et al. [48]
bFGF PDGF-BB IGF-1	In vitro	Canine tendon fibroblasts were cultured with fibrin matrices infused bFGF	Flexor digitorum profundus	Rabbit	Administration of IGF-1 (100 ng/mL), PDGF-BB (50 ng/mL) and bFGF (5 ng/mL) induced an increase in proliferation of up to 588 % bFGF induced a 2-fold increase in Col-I expression	Costa et al. [49]
bFGF	In vivo	Cells from rabbit flexor digitorum profundus tendon were cultured with IGF-1, PDGF-BB and bFGF synergistically	Achilles	Rat		Liu et al. [50]
bFGF	In vivo	Rat Achilles tendons were repaired using electrospun PLLA scaffolds with or without bFGF nanoparticles	Superficial digital flexor	Rabbit	bFGF treatments induced a 55 % reduction in adhesions and enhanced maximal stress, yield stress, stiffness, ultimate strain, and yield strain	Moshiri et al. [51]
bFGF	In vivo	Rabbit SDFT tenotomy and repair followed by and three bFGF injections (1200 ng/kg)	Superficial digital flexor	Rabbit	bFGF significantly enhanced maximal stress, yield stress, stiffness, ultimate strain, and yield strain	Oryan et al. [52]
bFGF	In vivo	Rabbit SDFT tenotomy and repair followed by and three bFGF injections (1200 ng/kg)	Superficial digital flexor	Rabbit	bFGF reduced adhesion formation and significantly enhanced maximal stress, yield stress, stiffness, ultimate strain, and yield strain	Oryan et al. [53]
bFGF	In vitro and in vivo	Severed rabbit tendons were repaired using gel-coated nylon sutures soaked in a 400 µg/mL bFGF solution	Intrasynovial flexor	Rabbit	The nylon thread absorbed up to 3 µg of bFGF in the 400 µg/mL solution. The bFGF thread increased proliferation of cultured tenocytes. In vivo, bFGF increased epitendon and	Hamada et al. [54]

Table 1 (continued)

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
bFGF	In vivo	Insertion point supraspinatus tendon ruptures were repaired with bFGF-treated fibrin sealant	Supraspinatus	Rat	epitenocyte proliferation and enhanced ultimate failure load by over 33 % at 3 weeks. There were no significant differences at 6 weeks Rats treated with bFGF had significantly higher tendon-to-bone insertion maturity scores and mechanical strength after 2 weeks than rats receiving untreated fibrin sealant, but there were no differences at four or 6 weeks	Ide et al. [55]
bFGF	In vivo	Insertion point rotator cuff repair using electrospun bFGF-enhanced PGLA scaffolds (20 µg/mL)	Rotator cuff	Rat	bFGF + PGLA significantly enhanced collagen organization, ultimate failure load, ultimate stress, and stiffness after 8 weeks relative to PGLA alone	Zhao et al. [56]
bFGF	In vivo	Rotator cuff repair with acellular dermal matrix graft and fibrin sealant infused with bFGF (100 µg/kg)	Rotator cuff	Rat	bFGF fibrin sealant improved tendon maturity scores and ultimate failure loads at six and 12 weeks relative to the unaugmented sealant	Ide et al. [57]
bFGF	In vivo	Flexor tendon repair with fibrin sealant infused with bFGF (500 or 1000 ng)	Flexor	Canine	bFGF increased cell proliferation, inflammation, adhesion formation, neovascularization, and scar formation.	Thomopoulos et al. [58]
bFGF	In vitro	Tenocytes from rat synovial tendons were cultured and treated with an adenovirus vector to mediate bFGF gene transfer	Intrasynovial	Rat	Adenovirus mediated gene transfer significantly increased expression of bFGF, Col-I, and Col-III	Wang et al. [59]
bFGF	In vivo	Tendon suture repair followed by adenovirus mediated bFGF gene transfer	Flexor digitorum profundus	Chicken	bFGF adenovirus treatment increased ultimate strength at 2 and 4 weeks compared to the sham virus and repair-only groups	Tang et al. [60]
bFGF	In vivo	Achilles tenotomy treated with MSCs which had been induced to express bFGF by a lentivirus vector	Achilles	Rat	There were no significant differences in histological features or failure load between the lentivirus + MSC, MSC, and untreated controls.	Kraus et al. [61]
PDGF-BB	In vitro	Equine SDFT explants were cultured with PDGF (0, 1, 10, 40, or 100 ng/mL)	Superficial digital flexor	Equine	At 100 ng/mL, PDGF increased Col-1 expression by 60 % and decreased Col-III expression by 51 %	Haupt et al. [62]
PDGF-BB	In vitro			Canine		Sakiyama-Elbert et al. [63]

Table 1 (continued)

Growth factor	Study method	Study type	Types of tendon	Species	Results	References
		Canine tenocytes were cultured with fibrin matrices enhanced with PDGF at 0.125, 0.25, and 1.25 µg/mL	Flexor digitorum profundus		PDGF + fibrin significantly increased proliferation (approximately 2-fold) and Col-I production relative to the fibrin and control groups	
PDGF-BB	In vivo	Canine flexor tendon repair augmented with PDGF (100 ng) released through a fibrin-heparin delivery system	Flexor	Canine	PDGF treatment enhanced joint flexibility and rotation, but had no effect on tendon mechanical properties	Gelberman et al. [64]
PDGF-BB	In vivo	Canine flexor tendon repair augmented with PDGF (500 ng) released through a fibrin-heparin delivery system	Flexor	Canine	PDGF treatment enhanced tendon gliding and fibroblast proliferation, but had no effect on tendon tensile properties	Thomopoulos et al. [65]
PDGF-BB	In vivo	Canine flexor tendon repair augmented with PDGF (40 ng) released through a fibrin-heparin delivery system	Flexor	Canine	PDGF treatment enhanced DNA content, fibroblast proliferation, and Col-I expression	Thomopoulos et al. [66]
PDGF-BB	In vitro	Tenocytes from rat intrasynovial tendons were cultured and treated with plasmids containing PDGF c-DNA	Intrasynovial	Rat	PDGF gene transfer increased expression of PDGF-BB and Col-I	Wang et al. [67]
PDGF-BB	In vivo	Achilles tenotomy repair augmented by PDGF injections at 3 or 10 µg	Achilles	Rat	The 3 µg injection caused a roughly 2-fold improvement in maximum load after 7 days. The 10 µg injection caused a twofold improvement in maximum load at 21 days	Solchaga et al. [68]
PDGF-BB	In vivo	Ovine rotator cuff repair using bovine collagen matrix scaffolds seeded with rhPDGF at dosages from 0 to 500 µg	Rotator cuff	Ovine	At dosages of 75 and 150 µg, PDGF significantly improved ultimate failure load relative to suture-only controls	Hee et al. [69]
PDGF-BB	In vivo	Achilles transection repair using dip-coated PDGF-BB sutures	Achilles	Rat	At dosages of 1.0 mg/mL and 10.0 mg/mL, PDGF significantly lowered cross-sectional area and increased ultimate tensile strength	Cummings et al. [70]
PDGF-BB	In vivo	Ovine rotator cuff repair using bovine collagen matrix scaffolds seeded with rhPDGF at dosages from 0.1–3.33 µg/mL	Rotator cuff	Ovine	Ultimate failure loads were not significantly improved by PDGF treatments, but tendon stiffness and collagen organization were enhanced	Uggen et al. [71]

controls receiving untreated sponges [25]. The injection of 2.5 µg of GDF-6 (BMP-13) or 7 also increased tendon cellularity, thickness, and the rate of repair in a rat Achilles transection model [26]. Pending further research, this may be a promising treatment for tendon damage.

GDF-5, 6, and 7 have been investigated as augments for tendon repair due to their tenogenic specificity. Other growth factors in the bone morphogenic protein (BMP) family have osteogenic effects and are thus unsuitable for midsubstance tendon regeneration, although these growth factors may have applications for enthesis repair. Bone morphogenic protein-2 (BMP-2) at 100, 500, and 1000 ng/mL and BMP-7 at 100, 500, 1000, and 2000 ng/mL enhanced Col-I production in cultured tenocytes, and BMP-7 increased cell activity in a dose-dependant manner [27]. RhBMP-2 injections (at a dosage of 15 µg) appeared to promote the growth of enthesis-like tissue and enhanced ultimate failure load in a rabbit model of flexor tendon reattachment surgery [28]. BMP-2's ability to induce bone formation may also enhance traditional surgical techniques. Kim et al. found that fibrin gel supplemented with BMP-2 at 10 µg/mL induced bone formation in suture anchor holes during patellar tendon reattachment surgery in a rabbit model. Consequently, the anchor connections were stronger, resulting in higher load-to-failure compared with suture holes treated with BMP-2-seeded Col-1 gel or untreated controls [29]. Not all such studies have been promising; Thomopoulos et al. found that BMP-2 delivery (at dosages 0.344 and 0.688 µg/L) via collagen sponges or calcium phosphate matrix, as an augment for canine flexor tendon reattachment, did not reduce the formation of fibrous scar tissue, increase ultimate failure load, or promote enthesis regeneration [30]. The authors noted BMP-2 concentrations at the injury site would have been low due to the slow release kinetics from the collagen and calcium phosphate. Thus, although the treatment could have promoted healing long-term, it was ineffective during the 7-day critical window immediately post-surgery [30].

TGF-β1 has also been investigated as an augment for tendon repair, but the results have not been as promising. In vitro TGF-β1 supplementation at 1, 10, and 100 ng/mL was found to increase Col-II expression in cultured rat tendon stem cells [31] and Col.V and XII expression in mouse tenocytes [32]. Although scleraxis was upregulated, Col-I was not [32]. Klauss et al. found that augmentation of cultured rabbit endotenon-derived cells with 2 ng/mL TGF-β decreased Col-I expression and increased the production of Col-III [33]. Injection of 10 or 100 ng TGF-β1 did, however, increase failure loads and procollagen I and III expression in a rat Achilles defect model [34]. Analogous treatments with 5 ng TGF-β1 injections also improved tangent modulus and failure loads in rabbit patellar tendon resections [35].

Although basic science studies for these growth factors have been promising, there is a dearth of clinical data. Human trials would be needed to validate whether GDF-5, 6, and 7 augmentation may be valuable clinical options for midsubstance repair and whether BMP-2 may enhance enthesis regeneration.

Insulin-Like Growth Factor

Insulin-like growth factor (IGF-1) may also have implications for tendon regeneration. IGF-1 augmentation of equine tendon progenitor cells cultured in monolayer at 100 ng/mL increased proliferation rate, GAG synthesis [36], and Col-I and Col-III expression [37], and equine flexor tendon explants cultured in 250 ng IGF-1/mL increased DNA content by 31 %, GAG content by 29 %, and collagen synthesis by 72 % [38].

IGF-1 treatments repeatedly enhanced ECM synthesis in vivo as well. IGF-1 injections (at a dosage of 1 mg) to the patellar tendon enhanced collagen fractional synthesis rate by 25 and 100 % respectively in healthy human patients when compared with saline-treated control tendons [39, 40]. In animal models, enhanced ECM production was associated with improved mechanical properties of healed tendons. Lyras et al. found that rabbit patellar tendon defects treated with IGF-1 and TGF-β1 in combination delivered via a fibrin sealant saw significant increases in stiffness and ultimate failure load [41]. Witte et al. (2011) administered four to five 25 or 50 µg intralesional IGF-1 injections to racehorses suffering from superficial digital flexor tendonitis (SDFT). IGF-1 augmentation reduced echolucency in 23 of 26 subjects, although only 62 % were able to return to racing [42]. Dahlgren et al. found that horses receiving ten 2 µg rhIGF-1 injections over 10 days saw significant reductions in the size of collagenase-induced lesions, although there were no significant differences GAG content, Col-I and III ratios, or tendon strength between IGF-1-treated and control tendons [43].

IGF-1 has also been studied in conjugation with stem cell treatments. Tendon stem cells treated with IGF-1 maintained multipotency for up to 28 days and increased decorin and scleraxis expression [31]. Schnabel et al. treated horses with SDFT using mesenchymal stem cells (MSCs) cultured with or without IGF-1. Although both treatments improved histological scores relative to PBS-treated controls, there were no significant differences between the MSCs and IGF-1 + MSC treatments [44]. Thus, IGF-1 does not seem to enhance the reparative effect of stem cell treatments in vivo. Alternative growth factors may have a synergistic effect with MSCs and other stem cell treatments; this is a fertile area for future study.

Growth hormone (GH) has also been considered for the treatment of tendinopathies. GH increases IGF-1 expression; acromegalic patients (who produce excess GH), for example, display a 2.9-fold increase in IGF-1 expression

relative to GH-deficient patients. Musculotendinous collagen expression was elevated 1.7-fold in these patients, which the authors attribute to this excess IGF-1 production [45]. Doessing et al. demonstrated that subcutaneous GH injections (at dosages between 33.3 and 50 $\mu\text{g/kg/day}$ for 14 days) increased tendon Col-1 expression in healthy human patients 3.9-fold [46]. Furthermore, GH has been demonstrated to aid in bone growth and healing [47]. Andersson et al. hypothesized that intramuscular GH injections at a dosage of 2 mg/kg for 10 days could stimulate the repair of transected rat Achilles tendons; however, it was found that GH treatments neither increased the strength of repaired tendons nor augmented the healing process [47].

In summary, IGF-1 promotes tendon regeneration by increasing Col-I production, resulting in increased stiffness and failure loads. GH increases IGF-1 production, although it is unclear if it has applications in tendon regeneration due to a lack of evidence. Further research should determine whether GH has synergistic effects with IGF-1, or whether IGF-1 alone is a superior treatment.

Fibroblast Growth Factor

Basic fibroblast growth factor (FGF) (bFGF or FGF-2) has also been proposed as a treatment for tendon damage. In vitro tests have shown that bFGF delivered via fibrin matrices at dosages between 0.125 and 1.25 $\mu\text{g/mL}$ induced a 2-fold increase in canine tenocyte proliferation [48], and administration of bFGF (5 ng/mL), PDGF (50 ng/mL), and IGF-1 (100 ng/mL) to cultured synovial sheath, epitenon, and endotenon cells harvested from rabbit digital flexor digitorum profundus tendon increased proliferation by up to 588 % [49]. FGF-2 augmentation at 100 ng/mL of equine tendon progenitor cells cultured in monolayer significantly increased ECM and Col-III synthesis [37]. However, decreases in Col-I and III expression were observed in canine tenocytes, perhaps hindering repair [48].

In vivo studies have also been promising. In a rat model of Achilles repair, electrospun PLLA scaffolds with bFGF nanoparticles (releasing 2900 pg of bFGF) increased Col-1 expression 2-fold relative to controls implanted with empty scaffolds [50]. Three studies demonstrated that serial bFGF injections (three 1200 ng/kg injections administered over 1 week) also improved the biomechanical properties of repaired rabbit SDFTs [51–53]. In one study, adhesions were only present in 30 % of experimental tendons as opposed to 85 % of saline-injected controls after 28 days [51]. Experimental tendons in all three studies more closely resembled healthy tendon tissue, and experimental rabbits were more physically active than controls. Moreover, maximal stress, yield stress, stiffness, ultimate strain, and yield strain were significantly improved relative to controls [51–53]. Gel-coated nylon sutures soaked in a 400 $\mu\text{g/mL}$ bFGF

solution and used to treat severed rabbit flexor digitorum fibularis tendons (FDFTs) increased epitenon proliferation and epitenocyte infiltration and enhanced ultimate failure load by more than 33 % at 3 weeks, suggesting that bFGF may specifically promote early healing [54]. In accord with this result, Ide et al. found that rat supraspinatus tendon ruptures repaired with bFGF-treated fibrin sealant (100 mg/kg) had significantly higher tendon-to-bone insertion maturity scores (15.8 ± 0.8 vs. 10.6 ± 0.5 out of 32) and mechanical strength (6.6 ± 2.0 N vs. 3.2 ± 0.6 N) than tendons repaired with untreated fibrin sealant after 2 weeks, although there were no significant differences between the two groups at 4 or 6 weeks [55]. Alternative growth factor delivery systems, such as scaffolds, may enhance or prolong bFGF's healing potential. Zhao et al. found that bFGF-seeded electrospun, randomly aligned PGLA scaffolds (at a dosage of 20 $\mu\text{g/mL}$), significantly improved collagen organization at all time points (22.6 ± 0.7 gray-scale units at 2 weeks, 32.7 ± 0.8 at 4 weeks, and 45.4 ± 1.2 at 8 weeks vs. 20.5 ± 0.9 , 31.4 ± 0.7 , and 43.8 ± 1.0 for scaffolds without bFGF), ultimate failure load at 4 and 8 weeks (21.4 ± 1.3 N and 32.7 ± 1.0 N for the PGLA + bFGF vs. 20.7 ± 1.6 N and 28.4 ± 1.2 N for PGLA), ultimate stress at 8 weeks (1.82 ± 0.03 vs. 1.62 ± 0.03 MPa), and stiffness at 8 weeks (14.9 vs. 13.7 N/mm) in a rat model of rotator cuff repair [56], and administration of a fibrin sealant with bFGF (100 $\mu\text{g/kg}$) in conjugation with acellular dermal grafts significantly improved tendon maturity scores (24.3 ± 1.0 vs. 20.6 ± 2.6 out of 28 at 6 weeks, 26.7 ± 0.8 vs. 25.2 ± 0.5 at 12 weeks) and ultimate failure loads (10.2 ± 3.1 N vs. 7.2 ± 2.2 N at 6 weeks, 15.9 ± 1.6 N vs. 13.2 ± 2.0 N at 12 weeks) relative to the graft + fibrin group in a rat model of rotator cuff repair [57].

Although bFGF has been shown to promote healing, Thomopoulos et al. found it to be harmful. FGF-2 (500 or 1000 ng) delivery via fibrin matrices in a canine model of operative flexor tendon repair not only increased cell proliferation, but also promoted inflammation, adhesion formation, neovascularization, and scar formation relative to controls only receiving operative repair. Tendons treated with 1000 ng bFGF had a mean Col-I/Col-III ratio of 3.4, whereas the operative repair group had a mean Col-1/Col-III ratio of 4.3 [58]. Zhao et al. [56] demonstrated that bFGF delivered at high doses via fibrin matrices can enhance tendon repair in a rat model; the findings of Thomopoulos et al. may indicate that bFGF has adverse effects that are more pronounced in a canine model.

Gene therapy has also been used to upregulate bFGF expression. In vitro adenovirus-mediated bFGF gene transfer increased expression of bFGF, Col-I, and Col-III in cultured rat tenocytes [59]. Tang et al. injected the severed ends of surgically repaired chicken FDFTs in vivo with an adenovirus vector to express bFGF. Ultimate strength of experimental tendons was significantly higher than controls receiving a sham virus or suture repair at 2 and 4 weeks. At 8 weeks, the ultimate strength of adenovirus + bFGF tendons was

84.8 ± 22.0 N, whereas the strength of repair-only tendons was 56.7 ± 17.6 N [60].

Not all gene therapy treatments were effective. Kraus et al. treated severed rat Achilles tendons with MSCs that had been induced to express bFGF via a lentivirus vector. The treatment was only marginally effective; it did not enhance failure load or stiffness, and no significant histological differences were seen between the bFGF, the sham virus, or untreated control groups after 4 weeks [61]. This indicates that direct injection of virus vectors, rather than indirect application via treated MSCs, is a more effective application of gene therapy.

Platelet-Derived Growth Factor

In vitro tests have shown that platelet-derived growth factor-BB (PDGF-BB) may augment tendon repair [62–66]. PDGF (100 ng/mL) increased Col-I expression by 60 % and decreased Col-III production by 51 % in equine SDFT explants [62], and PDGF (0.125, 0.25, and 1.25 μ g/mL) delivered via fibrin matrices induced an approximately 2-fold increase in proliferation and significantly increased Col-I production of cultured canine tenocytes at dosages of 0.125 and 1.25 μ g/mL [63]. Cultured rat tenocytes treated with 15 μ g of plasmid containing PDGF-BB cDNA underwent a 125 % increase in Col-I expression [67].

These benefits have been reflected in vivo; in three studies, rhPDGF augmentation (100, 500, and 40 ng respectively) of canine flexor tendon repair using a fibrin-heparin delivery system promoted joint flexibility and rotation ($19^\circ \pm 10^\circ$ for proximal interphalangeal joints receiving repair + PDGF vs. $8^\circ \pm 4^\circ$ for joints receiving repair only) [64], tendon gliding [65], fibroblast proliferation [65, 66], DNA content (20 % increase at 7 days), and Col-I expression at 7 days [66]. However, these treatments did not significantly affect the tensile properties of treated tendons and may not affect risk of reinjury [64–66]. The use of rhPDGF-BB demonstrated modest benefits in a rat model. In a rat model of Achilles tendinopathy, rhPDGF injections significantly increased maximal strength of augmented tendons compared with those treated with saline or PRP. The 3- μ g dose increased maximal load to 24.5 ± 4.3 N after 7 days, whereas maximal load of saline-injected controls was 12.5 ± 2.9 N. After 21 days, maximal load for tendons receiving 10 μ g PDGF-BB was 30.1 ± 5.5 N compared to 15.7 ± 4.4 N for controls [68]. In an ovine model of rotator cuff repair, bovine collagen matrix scaffolds seeded with rhPDGF at dosages of 75 and 150 μ g significantly improved ultimate failure loads (1490.5 ± 224.5 N and 1486.6 ± 229.0 N respectively vs. 910.4 ± 156.1 N for suture-only controls) [69]. Dip-coated PDGF-BB sutures (at dosages of 1.0 and 10.0 mg/mL) significantly lowered cross-sectional area and increased ultimate tensile strength (1.9 ± 0.5 and 2.1 ± 0.5 MPa respectively vs. 1.0 ± 0.2 MPa for controls) in a rat model of Achilles repair [70]. PDGF sutures did not significantly improve tendon

strength in an analogous study investigating ovine rotator cuff repair using PDGF-coated sutures at concentrations ranging from 0.1 to 3.33 mg/mL, but tendon stiffness and collagen organization were enhanced at all concentrations, resulting in a tendon–bone attachment more closely resembling an enthesis structure (Fig. 1) [71].

Although PDGF-BB enhances proliferation and Col-I expression, there is conflicting evidence that this treatment substantially affect tendon tensile properties. Any adverse side effects of PDGF-BB may have been more pronounced in the canine model, resulting in substandard repair relative to PDGF treatments in rat and ovine models. PDGF will need to be studied in a clinical setting to determine whether it is suitable for humans. Furthermore, PDGF dip-coated sutures promoted repair of insertion-site injuries in rat and ovine models. Further research should examine the role of PDGF in enthesis regeneration.

Platelet-Rich Plasma

Platelets contain many growth factors and thus can promote healing through similar mechanisms as growth factor applications [10]. However, the platelet concentration in PRP is relatively low, only two to ten times more than that of whole blood. For this reason, the growth factor concentrations released from PRP are analogous to those of the body, reducing the risk of complications. Dallaudière et al. [72] treated 408 patients with intratendinous PRP injections. During the 32-

Fig. 1 Sheep enthesis 6 weeks post-operatively. Insertion point defects were repaired with two suture anchors using rhPDGF-BB-coated sutures. Tissue sections were stained with Safranin-O/Fast Green ($\times 100$ magnification). Distinct tendon (T), cartilage (C), and bone (B) layers are evident in the sample, suggesting this treatment may promote enthesis regeneration [71]

month trial, no clinical complications were reported, demonstrating the safety of such procedures.

In vitro studies have shown PRP to be a promising treatment. Beitzel et al. showed that PRP significantly increases tenocyte viability when compared with an FBS-treated control [73]. Sadoghi et al. treated human tenocytes with PRP of varying platelet concentrations (1-, 5-, and 10-fold). All PRP treatments increased proliferation, DNA levels, and GAG levels, although lower concentrations were more effective [74]. Jo et al. found that PRP significantly increased the production of matrix, GAGs, and Col-1 and III in human tenocytes in a dose-dependant manner [75].

PRP for Acute Tendinopathies

Animal studies have demonstrated the efficacy of PRP treatments for the healing of acute tendon tears or ruptures. Lane et al. treated damaged rabbit patellar tendons with PRP injections. It was found that the injections resulted in hypercellularity, an upregulation in Col-1 expression, and an increase in cell proliferation rate [76]. PRP treatments in combination with low-level laser therapy could also enhance the production of Col-1 in a rat model, whereas unaugmented laser therapy had no significant benefits [77]. PRP administration of rat Achilles ruptures also enhanced Col-1 expression and ultimate failure loads when compared with controls given saline or platelet-poor plasma treatments [78, 79]. The PRP treatments also increased Col-1 fiber density and inflammation [79].

In randomized human clinical studies, PRP injections were found to significantly reduce pain [80–82], increase shoulder rotation, and improve load-bearing capacity [80, 81] during the first 3 to 6 months after rotator cuff surgery, suggesting that PRP may aid early healing. Moreover, PRP was found to significantly reduce retear rates in the long term [83]. De Almeida et al. similarly found that PRP significantly reduced the size of the patellar tendon gap area after ACL reconstruction, signifying a more complete repair [80]. Sanchez et al. found that augmentation of Achilles suture repair using platelet-rich fibrin matrices also improved range of motion, decreased tendon cross-sectional area, and shortened recovery, allowing athletes to resume training [84].

Other studies did not find PRP to be effective. PRP did not affect the biomechanical properties of Achilles repair in a rat model [68], and PRP-treated flexor-tendon lesions in sheep were found to have elevated Col-III expression, poorly aligned ECM, and extensive, pathological hypervascularization [85]. Beck et al. surgically repaired defects of the supraspinatus tendon–bone interface using transosseous repair methods in a rat model. The PRP group tendons were also augmented with subsequent PRP injections. After 21 days, there were no significant differences between the repair and PRP groups in failure load, although the PRP group had a higher failure strain and the standard repair group had higher stiffness. PRP collagen fibers were

more organized and thicker than in the control. Aside from slight infiltration by hypertrophic chondrocytes during the first 7 days, the PRP treatment did not have any negative effects but did not aid or accelerate healing [86].

In a human clinical study, PRP augmentation of Achilles rupture repair did not improve modulus or tendon function (as measured using a heel-raise index) after 1 year [87]. This may suggest that PRP is less effective at healing more severe injuries.

To date, the results of PRP on acute tendon repair have been conflicting at best. There are many confounding variables within these clinical studies including the mechanism PRP delivery, white blood cell concentration, and even the daily fluctuations of growth factor and platelet levels within a single individual. This variation may significantly affect outcomes.

PRP for Chronic Tendinopathies

Outcomes following PRP treatments for chronic tendon injuries and inflammation have also been mixed. Over eight clinical human trials, the vast majority of patients suffering from chronic, recalcitrant tendinosis saw significant and substantial reductions in pain [88–95] and increases in joint function [72, 88, 90, 92–95] when treated with PRP without supplemental procedures. These patients had experienced symptoms of tendinosis for at least 3 months and had not responded to nonsurgical interventions [88–90, 92–95]. Raymond Monto treated 30 patients experiencing severe, persistent Achilles tendinopathy with a single 4 mL injection of PRP [90]. After 2 years, mean pain scores had improved dramatically. Nine of ten patients who had been forced to leave their jobs due to their injuries were able to return to work, and 18 of 22 treated athletes were able to resume playing their sports. In a similar study, 32 patients suffering from midsubstance Achilles tendinosis were treated with a single PRP injection [91]. Within 6 months, 25 patients were asymptomatic.

Despite marked improvements in tendon pain and function after PRP treatment, its effects may not be as substantial as previously suggested. In human clinical studies, de Vos et al. found that PRP did not significantly affect ultrasonographic tendon structure, neovascularization, or pain scores compared with saline placebo controls [96, 97]. A 1-year follow-up confirmed that PRP did not affect clinical outcomes or the duration of injury recovery [98]. Jo et al. demonstrated that PRP did not diminish pain in patients after rotator cuff repair surgery. Moreover, although the PRP group did experience a lower retear rate than controls, this difference was not statistically significant [99]. Owens et al. found that MRI appearance improved for only one of six damaged tendons treated with PRP, suggesting they had not healed properly [92]. However, despite lack of apparent healing, pain and function scores rose significantly. Raeissadat et al. also found that PRP was no

more effective as whole blood injections. Thirty patients received 2 mL of whole blood, and 31 received PRP. Both groups saw significant reductions in pain, but there were no significant differences in the outcomes of either group [93].

Many patients whose symptoms were relieved by PRP therapies may have seen improvements without treatment. In a double-blinded, controlled, randomized study involving 230 patients, Mishra et al. demonstrated that PRP can reduce symptoms of tennis elbow [89]. The PRP group had a success rate (defined as a 25 % reduction in pain) of 84 % over 24 weeks. However, controls treated with bupivacain (a local anesthetic) had a success rate of 64 %. This difference was significant, but it suggests that PRP only improved prognosis by 20 %. Regardless, PRP-treated patients did experience significantly larger reductions in pain than the control group, indicating that, although the treatment is not as effective as suggested in other studies, it is still beneficial for patients.

PRP has also been demonstrated to be effective in conjunction with other techniques for the treatment of tendinosis [100–102]. A treatment involving the application of adipose-derived MSCs (ADSCs) and PRP to alleviate digital flexor tendonitis in horses was able to return 17 of 19 horses to their previous levels of competition [102]. Only two horses experienced reinjuries. However, as PRP and ADSC treatments were not evaluated separately, it is not clear which augments had a larger impact on the healing process. In a human clinical study, Finnoff et al. treated 31 patients suffering from tendinosis with needle tenotomy supplemented with a PRP injection [101]. Eighty-four percent of patients regained tendon function, and 86 % of patients experienced significant reductions in pain. In a double-blinded, randomized, controlled trial, Dragoo found that dry-needling (DN) treatments are enhanced by supplementation with PRP [100]. Every patient treated with PRP saw improvements in their symptoms, whereas three of 13 DN treatments failed. Moreover, patients receiving PRP injections recovered more rapidly and had significantly higher VISA (Victorian Institute of Sports Assessment) scores for tendinopathy at 12 weeks.

PRP may be more effective than some traditional techniques. Smith et al. demonstrated that PRP treatment had better functional outcomes than electroshock wave therapy (ESWT) [95]. In a clinical trial, both procedures reduced pain and improved VISA scores; however, VISA scores of PRP-treated patients were significantly higher than those of ESWT patients after 6 and 12 months, and 91 % of PRP patients were satisfied with the results of their procedures, as opposed with only 61 % of ESWT patients.

There is mixed evidence supporting the use of PRP for chronic tendinopathies. Multiple animal studies and clinical trials can reduce pain and increase tendon function when traditional treatments have failed. However, several studies have shown that PRP had no effect on patient outcomes [96–99]. These conflicting data may be caused by variations in PRP

composition, such as platelet and white blood cell concentrations. Future research should strive to identify the ideal PRP makeups to enhance tendon repair. Given the marked improvements in pain and tendon function scores after PRP treatment reported by some studies, PRP has the potential to be an effective, cheap, and safe procedure if the ideal composition can be identified.

Stem Cells

Bone Marrow Stem Cells

The ability of mesenchymal stem cells (MSCs) to augment tendon healing has been demonstrated in both animal and human trials. Injections of MSCs into midsubstance Achilles tendon ruptures increased ultimate failure load in a rat model [103, 104]. Huang et al. found that failure load for the normoxic MSC group was 2.7 N/mm² at 4 weeks vs. 1.7 N/mm² for untreated controls. Culturing MSCs in a hypoxic environment (1 % O₂) significantly more than doubled failure load (5.5 N/mm²) relative to normoxic MSCs [103]. Okamoto also found MSCs to be more effective than bone marrow cells (BMCs). Ultimate failure loads following MSC treatment were 3.8 N vs. 0.9 N for controls and 2.1 N for bone marrow cell-treated tendons ($p < 0.016$) [104]. Nourissat et al. [11] removed the enthesis of the Achilles tendon in a rat model. Two tunnels were made through the calcaneum, the detached tendon was sutured to the bone, and the injury site was then injected with either chondrocytes, MSCs, or saline. The MSC and chondrocyte-treated tendons achieved significantly higher failure loads after 45 days (84.6 ± 17.1 N and 80.3 ± 13.0 N respectively) than the saline group (68.6 ± 15.1 N) and, in fact, had higher failure loads than healthy, uninjured tendon (74.4 ± 10.9 N). Moreover, the MSC group regenerated an organized enthesis, showing that MSC treatments can result in high quality repairs [11].

Human clinical trials investigating the repair of rotator cuff tears using MSCs have also been promising [105, 106]. Hernigou et al. found that a bone marrow aspirate concentrate (BMAC) injection, in conjunction with suture-anchor repair surgery, significantly lowers the risk of retears of the supraspinatus tendon after surgery. Twenty-five of 45 control group patients experienced tendon retears within 10 years of the repair procedure; in contrast, only 6 of 45 patients who received BMAC injections experienced analogous reinjuries [105]. In fact, patients in the BMAC group who had received fewer MSCs were significantly more likely to have a retear. Ellera Gomes et al. [106] complemented the use of sutures with injected bone marrow mononuclear cells (BMMCs) for rotator cuff lesion repair in 14 patients. After 1 year, the integrity of the tendon tissue in all patients remained

uncompromised, and mean UCLA shoulder rating scores increased from 12 ± 3.0 to 31 ± 3.2 .

Ozasa et al. found that bone marrow stem cell (BMSC) and muscle-derived stem cell (MDSC) treatments promoted tendon healing in excised canine Achilles, resulting in regenerated tissues with similar mechanical properties [107]. There were no significant differences between the two groups in mean failure strength or mean stiffness after 4 weeks. However, MDSC administered in conjugation with a 100 ng/mL rhGDF-5 gel patch significantly enhanced mean failure strength relative to the BMSC ($p < 0.001$) MDSC ($p < 0.001$) and BMSC + GDF-5 tendon ($p = 0.019$). No clinical studies have been conducted on MDSCs; this may be a promising future direction for stem cell research. This study also indicates that stem cells may have synergistic effects with growth factors.

Adipose-Derived Stem Cells

ADSCs are an alternative to bone marrow-derived MSCs that can be harvested using liposuction. ADSCs release growth factors that may promote differentiation and immunosuppressive factors that may reduce inflammation [108, 109]. Moreover, they require much less invasive procedures to obtain than MSCs. Therefore, ADSCs may be a viable alternative to MSCs. Using a rabbit model, Oh et al. [110] found that the injection of cultured ADSCs into muscle adjacent to the insertion site of torn subscapularis tendons in conjugation with a standard suture anchor treatment resulted in a higher quality repair than the suture anchor repair alone. The ADSC-treated tendons had less fatty infiltration, stronger tendon–bone connections, and higher load-to-failure values than the saline + suture anchor repair tendons. Uysal et al. similarly found that ADSC augmentation of rabbit Achilles repair significantly improved tensile strength of the regenerated tissues [111]. Future studies directly comparing the effects of MSC and ADSC administration will be necessary to determine whether ADSCs are a viable replacement for MSCs.

Periosteum

Periosteal cells can differentiate into either chondrocytes or osteocytes, and they may promote enthesis regeneration. Chang et al. detached the infraspinatus tendon from the greater tuberosity and sutured periosteal flaps from the proximal tibia to the torn tendon in a rat model. Controls received the same treatment without periosteum augmentation. Extensive fibrocartilage and bone had formed at the tendon–bone interface of the experimental group after 12 weeks, perhaps indicating the regeneration of an enthesis. This was accompanied by significant increases in failure load and attachment strength [112].

Karaoglu et al. compared periosteum and bone marrow aspirate treatments in rabbit extensor digitorum repair. The tendons of the periosteum group were wrapped in freshly harvested periosteal tissue, and a BMAC group was given a BMAC injection at the injury site. The control group underwent analogous surgeries without the inclusion of BMAC or periosteum. The periosteum and BMAC groups had thinner, better-organized tendon tissue after 6 weeks. Obvious bone ingrowth was present in both experimental groups. After 12 weeks, a well-defined fibrocartilage zone was only present in the BMAC group. Overall, it appeared that periosteum had an early repair advantage [113].

Cell-Seeded Scaffolds

Scaffolds have been shown to improve the mechanical properties of repaired tendons [114–121], so several studies have investigated artificial scaffolds and stem cell treatments in conjugation. Kim et al., investigating the viability of seeded stem cells, repaired full-thickness window defects of the infraspinatus tendon of 50 rabbits with MSC-seeded or unseeded open-cell PLA fiber scaffolds. After 6 weeks, fluorescence microscopy showed an increase in cell density in the seeded scaffolds, and the production of Col-I was significantly higher in the seeded scaffolds [122].

Funakoshi et al. investigated the efficacy of a fibrocyte-seeded, chitosan-based hyaluronan hybrid polymer fiber scaffold (CSS). A defect of the humeral insertion of the infraspinatus tendon was repaired with either fibroblast-seeded or unseeded CSS scaffolds in a rabbit model. After 12 weeks, the regenerated tissue of the seeded CSS group had a significantly higher tangent modulus and tensile strength than the repairs involving unseeded scaffolds. Moreover, Col-I production was only seen in the fibroblast-seeded scaffolds [123].

Yokoya et al. found that MSC-seeded poly(lactide-co-glycolide) (PGLA) fiber scaffold rotator cuff repairs had significantly higher tensile strength (3.04 ± 0.54 , 2.38 ± 0.63 , and 1.58 ± 0.13 MPa for MSC, PGLA, and control groups, respectively, at 16 weeks), failure loads (111.9 ± 9.43 , 90.0 ± 11.3 , and 44.3 ± 3.67 N at 16 weeks), and maturity scores (21.0 ± 0.82 , 16.7 ± 2.05 and 10.2 ± 0.98 at 8 weeks) than both the unseeded PGLA group and controls receiving no scaffold in a rabbit model. Both the seeded and unseeded scaffolds were comprised primarily of Col-III at 8 weeks; after 16 weeks, Col-I/Col-III ratios had improved in the MSC-PGLA tendons, but not in the PGLA-only group [124].

Biological scaffolds may have advantages over absorbable synthetic scaffolds, as they may better mimic the environment of uninjured tendon, guiding the differentiation of seeded cells. Fini et al. found that rat tenocytes seeded on decellularized human dermis produced significantly more Col-I than tenocytes cultured in polystyrene wells ($p < 0.0001$ at 3 days)

[125]. These results indicate that biological, ECM-derived scaffolds may promote the production of Col-1, leading to higher quality repairs. However, one study found that biological and artificial scaffolds could produce in comparable mechanical properties. Pietschmann et al. treated midsubstance defects of rat Achilles tendon with polyglycolic acid (PGA) fiber or porous Col-I scaffolds seeded with either MSCs or tenocytes. There were no significant differences in failure strength or failure strength/cross section between the PGA and Col-I scaffolds. MSC-seeded PGA and Col-I scaffolds were no more effective than unseeded controls, potentially due to MSC-induced ossification. However, the tenocyte-seeded scaffolds had significantly higher failure strength/cross section than either the MSC or empty scaffolds [126].

Little et al. investigated whether ligament-derived matrix (LDM) could induce human ADSCs to express a ligamentous or tendinous phenotype. Pulverized anterior cruciate ligament harvested from porcine knee joints was mixed with rat tail Col-1 to form the LDM gel scaffolds. Human ADSCs were cultured on these scaffolds or on rat Col-I scaffolds without porcine ligament. ADSCs seeded on the LDM scaffolds demonstrated elevated GAG content and Col-I and Col-III synthesis relative to the Col-I scaffold controls. The LDM also increased cell proliferation, and the authors concluded that the treatment had induced the ADSCs to express a tendinous or ligamentous phenotype. Chainani et al. further highlighted potential benefits of ECM scaffolds. Electrospun polycaprolactone (PCL) scaffolds were coated with either fibronectin (FN), phosphate-buffered saline (PBS), or tendon-derived ECM (TDM) and then seeded with ADSCs. Col-III expression increased at day seven after culturing for all coating types, and Col-1 expression began increasing after day 4. However, Col-1 content was significantly elevated in the TDM scaffolds after 28 days [127].

In summary, scaffolds augment the mechanical strength of healing tendons and may guide the differentiation and ECM production of stem cells, leading to repairs more closely resembling natural tendon. ECM-derived scaffolds induce Col-I expression and may therefore be more suitable than synthetic scaffolds when administered in conjugation with tenocytes and stem cells. There is little data regarding how scaffold architecture (rather than composition) directs stem cell or fibroblast phenotype; this should be addressed with further research. Furthermore, clinical trials will be necessary to determine if these treatments are effective in humans and identify any adverse effects.

Conclusion

Biologics for tendon repair represent an attractive augmentation to traditional surgical methods. However, tendon healing and regeneration is an intrinsically complex process involving

numerous different growth factors. The process is a sequence of healing phases in which different growth factors and cells may be activated in disparate quantities. While research has illuminated many of the biochemical reactions in healing, the regenerative process of normal tendon remains elusive.

Growth factors have been shown to improve the quality of tendon repairs. The efficacy of GDF-5, IGF-1, and bFGF has been demonstrated in multiple studies, although clinical data on GDF-5 treatments is necessary. PDGF-BB increased ECM production but did not consistently improve tensile properties.

Evidence on the efficacy of PRP is mixed. However, there is good basic science supporting its use, and numerous clinical studies have found that PRP substantially reduces pain and increases joint function for chronic and acute tendinopathies. The standardization of PRP may enhance these effects. Moreover, reactions to autologous PRP are very uncommon. Therefore, the use of PRP may be warranted in patient refractory to other conservative treatments.

Cell-seeded scaffolds result in much higher quality repairs than unseeded controls or cells alone. The scaffolds increase mechanical strength of regenerated tendon tissues and promote the production of Col-I over Col-III. Tenocytes may be especially effective for midsubstance repairs, although stem cells would be required to regenerate a multilayered structure such as the enthesis.

Enthesis regeneration is one of the greatest challenges in the field of orthopedics today, but several growth factor and stem cell augments have proven promising. BMP-2 [28, 29], bFGF [56], and PDGF-BB [70, 71] all increased the strength and enhanced the tensile properties of repairs at the tendon insertion site, and BMP-2 and PDGF-BB administration resulted in regeneration of an enthesis-like structure [28, 70]. MSC [11] and BMAC [113] administration for insertion-site defects also resulted in multilayered, organized enthesis structures, and periosteal cells were similarly effective due to their ability to differentiate into osteocytes or chondrocytes.

Ultimately, as the regenerative puzzle is solved, it is likely that a combination of approaches will provide more advanced and successful outcomes. Stem cell research is particularly promising in that stem cells may mediate repair and continue to produce regenerative stimuli long after an initial application of a growth factors via a more conventional approach. The idea that stem cells may be small growth factor factories is appealing and may ultimately allow the tissues to autoregulate the appropriate chemical milieu at the various stages of healing to ensure more normal repairs. More research, particularly clinical research, that directly compares the efficacy of different cell types, growth factors, and materials used as augments is needed to reach that conclusion.

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