The role of growth factors in rotator cuff healing

Francesco Oliva, Alessio Giai Via, Nicola Maffulli *

Department of Orthopaedics and Traumatology, University of Rome "Tor Vergata" School of Medicine, Viale Oxford 81, Rome, Italy.

*Centre for Sports and Exercise Medicine Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Mile End Hospital, 275 Bancroft Road, London E1 4DG, UK.

Correspondence:

Nicola Maffulli, MD, MS, PhD, FRCS(Orth) Centre for Sports and Exercise Medicine Queen Mary University of London Barts and The London School of Medicine and Dentistry Mile End Hospital 275 Bancroft Road London E1 4DG, UK.

Tel: + 44 20 8223 8839

Fax: + 44 20 8223 8930

n.maffulli@qmul.ac.uk

Running title: Growth factors and tendon healing

ABSTRACT

The histological lesion underlying overuse rotator cuff tendinopathy is a failed healing response, with haphazard proliferation of tenocytes, disruption of tendon cells and collagen fibres, and increased non-collagenous extracellular matrix. Recent attention has focused on the biological pathways by which tendons heal, leading to the identification of several growth factors (GFs) involved in this process. No studies have been published on the time course of the various GFs during rotator cuff healing process *in vivo,* in humans. We review what is known about these GFs and their role in rotator cuff healing.

Keywords:

Growth factors; tendon healing; tendinopathy; review

Introduction

Rotator cuff (RC) tendon tears account for more than 4.5 million physician visits per year, and over 250,000 RC repair surgeries performed annually in the United States.[1] The pathogenesis of RC tears is debated.[2,3,4,5] The RC has limited ability to heal back to its insertion on the humerus following repair[6], possibly because of the poor vascularization of tendon tissue, and also because the histopathological changes which accompany a rupture are localized not only at the site of rupture but also in the macroscopic intact tendon portion, suggesting more generalised involvement of the tendon.[7,8,9] Gerber et al. reported a re-tear rate of 34% in 29 patients at an average of 37 months on MRI evaluation of the repair site.[10] Given this limited ability for healing[11], several strategies - including growth factors (GFs) and cytokines, gene therapy, tendon augmentation graft and tissue engineering with mesenchymal stem cells - have been proposed to enhance tendon healing.[12,6] Several GFs are upregulated during RC healing, and they may be used to augment rotator cuff repairs.[6]

The pathologic label 'tendinosis' has been in use for more than two decades to describe collagen degeneration in tendinopathy, which is only one of the features of tendinopathy.[13] Despite that, most clinicians still use the term “tendonitis” or “tendinitis”, thus implying that the fundamental problem is inflammatory. As the essential lesion of tendinopathy is a failed healing response, we advocate the use of the term “tendinopathy” as a generic descriptor of the clinical conditions in and around tendons arising from overuse, and suggest that the terms tendinosis, tendonitis and tendinitis only be used after histopathological examination.[13]

GFs are synthesized and secreted by a wide variety of inflammatory cells, platelets, fibroblasts, epithelial cells, and vascular endothelial cells. In a very specific fashion, they bind to external receptors on the cell membrane, which leads to intracellular changes in DNA synthesis and expression. In this manner, GFs directly affect cellular mitogenesis and chemotaxis and are able to influence the healing cascade. Large scale clinical trials have not been successful possibly through inadequate delivery strategies failing to meet the physiological requirements of the tissue repair

processes. These requirements are likely to comprise multiple GFs over a specific temporal pattern at an optimized ratio to meet the specific needs of the physiological process involved in tissue healing.[14,15] The exact process by which this happens remains to be clarified.[16]

Historical Studies

Several strategies have been proposed to enhance tendon healing and recently research has focused on regenerative therapies. Researchers have now been defining the growth factor requirements of tendon cells for over three decades. PDGF, EGF and insulin were the first growth factors applied in vitro to study the effect on tendon healing resulting in stimulation of tendon fibroblast proliferation.[17,18] This experimentation showed that EGF and insulin could reduce the reliance of the cultured tendon cell on serum. The positive impact of insulin and insulin-like growth factor (IGF-1) was again demonstrated in an *in vitro* rabbit flexor tendon model where matrix synthesis stimulation and increased cell proliferation were observed.[19]

In an alternative approach to GF supplementation, protein extracts were prepared from both normal and injured canine flexor tendons.[20] Through this methodology, it became apparent that normal tendons contained basic fibroblastic growth factor (bFGF), while platelet-derived growth factor (PDGF) and EGF were present in healing tendons. Leading on from this study, PDGF was demonstrated to promote repair of the medial collateral ligament of rats.[21] Bone morphogenetic proteins 7 (BMP 7) was excluded as a reparative GF in the rat's Achilles tendon repair model with bone formation observed.[22] Reinforcing the findings of previous *in vivo* studies, it was noted that low levels of bFGF were present in uninjured rabbit flexor tendons which then underwent upregulation following on from injury.[23] The technique of deriving platelet-rich plasma (PRP) was developed in the late 1990's,[24] but its clinical application is still debated in North America[25] and Continental Europe.[26,27]

Tendon repair

Tendon repair can occur either intrinsically via the resident tenocytes[28] or via extrinsic mechanisms, whereby cells from the surrounding sheath or synovium invade the tissue.[29] Three biologically and

temporally overlapping phases are described during tendon repair.[30] Various attempts to improve the healing process can have different effects on different phases. The healing process starts with:

1) The inflammatory phase: haematoma, platelet activation and invasion of cells that form a granuloma. Usually, this phase occurs three to seven days after the injury, cells migrate from the extrinsic peritendinous tissue such as the tendon sheath, periosteum, subcutaneous tissue, and fascicles, as well as from the epitenon and endotenon.[31]

2) In the formative phase, cells proliferate and differentiate. The migrated fibroblasts in the granuloma produce collagen (mostly collagen type III), which gradually increases its mechanical strength, so that loading can lead to elastic deformation, which allows mechanical signalling to start to influence the process. Production of collagen type I gradually takes over, and the repair callus reaches its largest size. The large transverse area compensates for tissue weakness, so that considerable traction forces can be sustained. Tenocytes become the main cell type, and over the next five weeks collagen is continuously synthesised. This phase of repair continues for eight weeks after the initial injury.[32]

3) Finally, in the remodelling phase, collagen type III is reabsorbed and replaced to produce better organisation, and cross-linking increases. Finally, the callus transverse area gradually decreases as the mechanical tissue properties improve. Despite intensive remodelling over the following months, complete regeneration of the tendon is never achieved. The tissue replacing the defect remains hypercellular. The diameter of the collagen fibrils is altered, favoring thinner fibrils with reduction in the biomechanical strength of the tendon.[33]

No studies have been published on the time of appearance of GF in the three different phases of the tendon healing process in humans. In a rabbit model, during healing of acute midsubstance rotator cuff tears, Kobayashi et al. assesed semiquantitatively the time expression of basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF) 1, platelet-derived growth factor (PDGF), and transforming growth factor (TGF-β) for 28 days. IGF-1 and TGF-β appear first in the blood cells in the inflammation phase; bFGF and PDGF appear later during the formative phase. TGF-β

was present for all the phases of the healing process, but the distal stump of the tear was lacking of these GF.[34] Galatz found a gradual increase of levels of TGF-β1 in a rat supraspinatus tendon model, which reaches a peak at 10 days.[35] Recently, Wurgler-Hauri et al[36] studied bFGF, BMP-12, BMP-13, BMP-14, cartilage oligomeric matrix protein (COMP), connective tissue growth factor (CTGF), PDGF-B, and TGF-β1 in tendon-to-bone healing in a rat supraspinatus model for 16 weeks. Immunoassays showed an increase in the expression of all GFs at 1 week, followed by a return to control or undetectable levels by 16 weeks in both the insertion and midsubstance. Of additional note in the insertion, COMP peaked at 1 week, followed by a decrease in expression at 2 weeks. BMP-12 and CTGF were moderately expressed across all time points. Furthermore, BMP-12 and PDGF-B were moderately expressed over time in the midsubstance.[36]

The release of GFs significantly increases the *in vitro* proliferation of human tendon cells and stimulates them to produce angiogenic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). This might be particularly relevant in the tendon repair process, assuming that the reduced blood supply to the tendon is associated with its low healing capability. Moreover, HGF is a potent anti-fibrotic agent with potential to reduce the formation of scarring around tendon tissue. Scarring itself is correlated with inferior repair quality.[37] Unfortunately, the experiments are not comparable, and only a small part of the whole process of tendon healing mediated by GFs is understood (Table I).

Growth factors in tendon healing

Numerous growth factors are involved in tendon repair. These include BMPs, EGF, FGF1, FGF2, IGF-I, IGF-II, PDGF-AA, PDGF-BB, PDGF-AB, TGF-β. These GFs may be produced locally by cells in areas of injury, growth and repair, or may be delivered by blood. In the rat supraspinatus tendon model, numerous GFs increased markedly 1 week after injury in both the insertion and midsubstance zones. These GFs included BMP-12, BMP13, BMP-14, bFGF, COMP, CTGF, PDGF-B, and TGFβ1. All increases in GFs had returned to pre-injury levels by 16 weeks establishing a clear connection between *in vivo* upregulation and repair.[36] Exogenous

supplementation of these factors in failed healing responses, such as in resistant tendinopathies, may lead to a definitive healing response.

Basic fibroblastic growth factor

bFGF is a single chain polypeptide of 146 amino acids, and is a member of the part of heparin-binding growth factor family. In humans, 22 members have been identified all of which are structurally related signaling molecules. It is angiogenic[38] and has mitogenic effects on many mesenchymal cells such as ligament fibroblasts.[39]

This growth factor has been shown to be involved in wound healing. An *in vitro* study show which bFGF has a stimulatory effect on human rotator cuff tendon cells but suppresses collagen synthesis.[40] Kobayashi *et al.*[34] show an immunohistochemical peak expression of bFGF in the first week in a midsubstance injury of supraspinatus tendon in the rabbit, and it was suggested that bFGF could be used to promote the healing process of a torn rotator cuff tendon.[6] In a rabbit supraspinatus tendon bone injury, Würgler-Hauri *et al.* supported this finding and, in addition, they detected another increase of bFGF at 8 weeks.[36] More recently FGF-2 has been studied in rat supraspinatus tendon models. Ide *et al*. found that the initial tendon-to-bone remodeling was accelerated by a local application of FGF-2 [41] and that the local application FGF-2 accelerates regeneration and remodeling of rotator cuff tendon defects reconstructed with acellular dermal matrix grafts.[42] The role of bFGF was also study by Chan et al.[43] which showed that, *in vitro*, supplementation of bFGF increases the proliferation of rat patellar tendon fibroblasts. Moreover Chang *et al.* showed that bFGF mRNA is upregulated in the tendon wound environment, and upregulated in tenocytes as well as in tendon sheath fibroblasts and inflammatory cells *in vivo.*[23]

Bone morphogenetic proteins

BMPs are a group of factors of the TGF-β superfamily that stimulate bone formation but also stimulate tendon cell mitogenesis and tendon healing. Although it is clear that BMPs stimulate tendon healing the mechanism remains unclear.[44,45] A combination of BMP signalling and influences of mechanical loading are likely crucial for tendon healing. This is reinforced by the

BMP-14 knockout mice studies where a delayed tendon healing response and irregular Type I collagen fibrils were observed.[45] However BMP-13, not BMP-14, appeared to be involved in early tendon healing, BMP-14 was primarily required for the maintenance of homeostasis of mature tendons, and BMP-12 was required for both.[46] BMP-12, BMP-13 and BMP14 have all been detected in intact human tendons by immunohistochemistry.[47,48] Ovoid tendon cells (tenoblasts) display elevated levels of both BMP-12 and BMP-13 when compared to the elongated tendon cells (tenocytes).[48] This suggests that tenoblasts are more active in matrix remodelling and healing than tenocytes. BMP-2, BMP-7, and BMP-12 all participate in tendon-bone healing and improve formation of new bone and fibrocartilage at the healing tendon attachment site, resulting in an improved load to failure of RC tendon.[49] Seeherman et al. demonstrated in a full-thickness rotator cuff tear sheep model that delivery of rhBMP-12 in sponge carriers had the potential to accelerate healing of rotator cuff repairs when compared to untreated controls.[50] In a sheep infraspinatus repair model BMP-12 determined increased formation of new bone and fibrocartilage at the healing tendon attachment site, and biomechanical testing showed improved load-to-failure.[51] Recently, gene therapy with the BMP-12 cDNA muscle graft showed histologically better organized and homogeneous pattern of collagen fibers at all time points than the control groups.[52]

Cartilage-derived morphogenetic protein

Cartilage-derived morphogenetic proteins-1 and -2 (CDMP-1 and CDMP-2) are members of the bone morphogenetic protein (BMP) family, which play important roles in embryonic skeletal development.[53] They are involved in skeletal development, joint morphogenesis and tendons formation. Its temporal and spatial expression pattern is mostly restricted to the developing appendicular skeleton.[54] In their study Nakase et al. found that CDMP-1 was synthesized by cells in the RC and which it was activated specifically at the site of the RC tear.[55] In an animal study Murray showed which CDMP-2-treated repairs of supraspinatus tendon tears were significantly stronger than the untreated repairs at 4 and 6 weeks post-operatively. The histological analysis also showed more organized healing.[56] CDMP-2 injection into transacted rabbit Achilles tendons

resulted in a 35% increase in mechanical strength 14 days post-operatively compared with controls.[57]

Insulin-like growth factor

Insulin-like growth factor, or IGF, is named for its hypoglycemic effect after intravenous administration. The stimulatory effects of IGF-I have been demonstrated in many cell types, including cartilage, bone, muscle and tendon cells.[58] During tendon healing, its role seems to stimulate the proliferation and migration of the tenoblasts during the inflammatory phase, while its increase in the remodelling phase seems to be clear.[59,60] In addition to the mitogenic effect, IGF-I can also stimulate selected components of matrix synthesis, and its expression was noted in avian tenocytes.[61] Complementary to this are the observations that IGF-I induced tenocyte migration, division, matrix expression and accelerated functional recovery from Achilles tendon injury in a rat model.[62,63,64]

Dines et al. are the only authors which investigated repair of rotator cuff tears using interposed genetically engineered autologous tendon in Sprague Dawley rats. Tenocytes were isolated from the rotator cuff and then transduced with the genes for PDGF-b or IGF-1. The transduction was performed using a retroviral vector. When incorporated into rotator cuff repair, the tendons transduced with PDGF-b showed no improvement over controls, and the fibroblastic cells transduced to express IGF-1 exhibited an improvement in both toughness and maximum load.[65] The expression of the insulin-like growth factor 1 binding proteins (IGFBPs) has also been studied in equine flexor tendons after acute injury and during healing over time: mRNA and protein expression for IGFBP-2, -3, and -4 was detected in normal tendon, and showed a marked increase following injury.[66] IGF-1, PDGF-BB, and bFGF were used alone and in combination to optimise tenocyte proliferation in cells of the synovial sheath, epitenon, and endotenon isolated from rabbit flexor digitorum profundus tendons. For all three tendon cell populations, proliferation at 72 h was greater in the presence of individual GFs. In addition, a synergistic effect was observed if GFs were

used in association as compared to maximal doses of individual GFs.[67] Systemic administration of IGF-I improves healing in collagenous extracellular matrices from loaded and unloaded tissues.[68]

Platelet-derived growth factor

PDGF was first isolated from platelets, but can be produced by many different cells, including smooth muscle cells.[59] PDGF is a basic protein of approximately 30 kD formed by two subunits (α and β chain) that exist in three isoforms. Most studies are focussed on the homodimer PDGF-BB isoform, which has stimulatory effects on both cell division and matrix synthesis, but only one used a rotator cuff model. In his tissue engineering study, Uggen used RC rat tendon fibroblasts transduced with either PDGF-BB or IGF-1. Encouraging results have been found in the experimental group receiving tissue construct grafts containing cells expressing PDGF-BB.[70]

An investigation into the consequences of administration of PDGF-BB directly into the wound gap of rat patellar tendons showed that early supplementation (day 3 post-injury) was not beneficial to restoration of mechanical properties,[71] probably from an increase in cell proliferation without matrix production. Later supplementation with PDGF-BB (on day 7 post-injury) did stimulate matrix production, with an accompanying higher peak load and pyridinoline content. PDGF-BB stimulated matrix and DNA synthesis in a dose-dependent manner in intrasynovial intermediate and proximal segments of deep flexor tendons, and extrasynovial peroneal tendons of rabbits during short-term cultures. PDGF-BB stimulated collagen synthesis and noncollagen protein synthesis in proximal intrasynovial tendon segments more than in extrasynovial peroneal tendon segments, and DNA synthesis less in proximal than in intermediate intrasynovial tendons.[72]

PDGF holds particular promise in combination with other GFs. Tendon cells express the receptor for PDGF, but do not normally express PDGF itself.[61] When applied with IGF-1, robust stimulation of tendon fibroblast migration and cell division are produced.[62,63,73] In addition, IGF-1 and PDGF act synergistically with cyclic tension to stimulate cell division. A further examination into the response of human tendon fibroblasts to cyclical mechanical stretching demonstrated that concentrations of TGF-β, bFGF and PDGF all increased compared to non-stretched controls.[74]

Moreover, serum, which contains both PDGF and IGF-I, stimulates cells in the whole tendon both mitogenically and matrigenically, and synergistically with cyclic load.[75] Taken together, these suggest that PDGF and IGF-1 may exert a positive influence on tendon and ligament healing through stimulation of cell proliferation, differentiation and matrix formation. Additional studies have shown that it is likely that matrix formation was stimulated predominantly by PDGF.[76,77] Uggen showed which PDGF-BB transduced cells stimulated adjacent rat tendon fibroblasts to increase collagen synthesis by 300% at 24 hours compared to a 28% increase in IGF-I transduced cells.[70]

In a study of intrasynovial canine tendon, PDGF-BB and bFGF significantly increased flexor tendon fibroblast proliferation, collagen production and matrix synthesis when each was applied on its own. The combined administration of PDGF-BB and bFGF led to increased proliferation.[78] In a study on the expression of GFs in normal canine flexor tendon healing, PDGF-AA, PDGF-BB and VEGF appeared in the whole tendon section at 10 days following tendon injury.[79] Mechanical stretching of tendon fibroblasts also promoted increased concentrations of TGF-β, PDGF and bFGF, suggesting that cyclical mechanical stretching may have a positive influence on tendon and ligament healing through stimulation of cell proliferation, differentiation and matrix formation.[74] PDGF in association with hypoxia exerts a synergistic effect which increases the expression of VEGF in Achilles tendon fibroblasts.[80]

In a similar canine model, PDGF-BB delivery increased cell proliferation, matrix remodelling, and accelerated flexor tendon healing.[81] The functional properties of repaired intrasynovial flexor tendons in an animal model study were significantly improved with the sustained administration of PDGF-BB. The range of motion of the proximal and distal interphalangeal joints was significantly higher for the PDGF-BB-treated tendons compared with the repair-alone tendons. Excursion values were also significantly higher in the PDGF-BB-treated tendons. There were no significant differences in tensile properties when comparing PDGF-BB-treated with repair-alone tendons. The

failure to achieve improvements in ultimate load, stiffness, and strain in the experimental group may have resulted from suboptimal PDGF-BB dosage or suboptimal release kinetics.[82]

Exogenous PDGF genes can be transferred effectively into intrasynovial tenocytes. The transfer increases significantly the expression of genes for PDGF and type I collagen.[83] In another study, PDGF gene therapy was more beneficial to tendon healing than VEGF gene therapy in an *in vitro* study of rat intrasynovial tendons.[84]

Transforming growth factor β

Originally, TGF-β was thought to be related to cellular transformation events prior to neoplastic growth. It is now clear that TGF-β has numerous physiological effects.[85,86] The expression of TGF-β appears closely tied to the expression of a differentiated phenotype in many cell lines including the mesenchymal precursor. Tendon and ligament formation has been tied directly to factors belonging to the TGF-β superfamily.[87] Proliferation, matrix synthesis and differentiation have also been affected in tenoblasts, chondroblasts and osteoblasts.[88] Whether this is an inhibitory or stimulatory effect depends on the stage of differentiation, presence of other GFs, and assay system used.[89,90] TGF-β is a weak stimulator of tendon cell migration and mitogenesis, but can stimulate robust expression of extracellular matrix.[91] TGF-β affects gene expression primarily through the activation of the Smad signaling pathway. The first step in the Smad pathway is the expression of TGF-β inducible early gene (TIEG). Healing of tendons in the TIEG knockout mouse suggests the possibility of tendon healing in the absence of the Smad pathway, and the existence of TIEG independent routes.[92] TGF-β1 significantly increased the amount of SMA (alpha-smooth muscle actin) in nonvascular cells in seven human rotator cuffs, suggesting that SMA-containing cells could contribute to the retraction of the torn ends of a ruptured rotator cuff and play an important role in healing.[93]

TGF-β may control the switching point in the healing process from normal to pathological.[94] All three TGF-β isoforms significantly increase collagen I and III production in cultured tendon

fibroblasts.[95] TGF-β1 induced a greater degree of contraction in tendon fibroblasts cultured in collagen gels as compared with TGF-β3.[96] This might explain the finding that TGF-β1 induces scar tissue formation, whereas TGF-β3 reduces it.[97] Intraoperative infiltration of neutralizing antibody to TGF-β1 improves flexor tendon excursion, but simultaneous infiltration of neutralizing antibody to TGF-β2 nullifies this effect. Therefore, TGF-β isoforms may interact with one another to modulate collagen synthesis in healing tendons.[98]

The temporal and spatial distribution of three TGF-beta receptor isoforms (RI, RII, and RIII) was analysed in a rabbit zone II flexor tendon wound healing model. This demonstrated that TGF-beta receptors were up-regulated after injury and during repair.[99] Receptor production was concentrated in the epitenon, tendon sheath and along the repair site. Peak levels of TGF-beta receptor expression where noted on day 14 and persisted until day 56.

The pathogenesis of tissue fibrosis during flexor tendon repair is also dependent, in part, on TGF-β signalling. In a rabbit flexor tendon model, TGF-β inhibition by neutralizing antibody was effective in reducing collagen I production in cultured flexor tendon cells which in turn could potentially reduce scar formation.[100] This suggests that, while TGF-β1 promotes scarring, TGF-β3 can improve the mechanical properties of healing tendons. Complementary to these findings were the observations concerning the locations of TGF-β1 and TGF-β3 and the formation of collagens in the rat supraspinatus tendon during tendon-to-bone healing after an acute injury. Collagen I protein and mRNA significantly increased at 10 days, and reached a plateau by 28 and 56 days. Collagen III showed a similar trend, with an early increase, remaining high until 56 days. TGFβ-1 was localized to the forming scar tissue and showed a distinct peak at 10 days. TGFβ-3 was not seen at the healing insertion site. Cell proliferation and density followed the same trend as TGFβ-1.[35]

Examination of tenoblasts from adult human patellar tendons revealed higher levels of TGF-β1 (and procollagen type I, Heat shock protein 47 (hsp47), MMP1, BMP12, BMP13) than tenocytes. Higher

expression of TGF-β1 might therefore be associated with the major activity of tenoblasts in tendon matrix remodelling .[48]

Formation of nitric oxide is an important event in the course of tendon healing, and its inhibition results in chronic inflammation and fibrosis due to an imbalance in TGF-β expression *in vivo*.[101] Examining the expression of inducible nitric oxide synthase (iNOS) and TGF-β in macrophage infiltrates within crush-injured digital flexor tendon and synovium of rats during normal tendon healing, the levels of TGF-β are high at first, and gradually decrease after 3 weeks of injury.[101]

Vascular endothelial growth factors

To our knowledge no studies on the use of VEGF for the treatment of RC tears are available in literature up today. VEGF are important signaling proteins involved in both vasculogenesis and angiogenesis. The broad term 'VEGF' covers a number of proteins from two families, that result from alternate splicing of mRNA from a single, 8 exon, VEGF gene. The most important member is VEGF-A, a glycosylated protein of 46–48 kDa composed of two disulphide-linked subunits. Other members are Placenta growth factor (PIGF), VEGF-B, VEGF-C and VEGF-D. The latter ones were discovered later than VEGF-A, and before their discovery VEGF-A was called just VEGF. All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation, although to different sites, times and extents.[102] Increased VEGF has been found rotator cuff tear. Vessel density and VEGF expression had significantly increased with extent of tendon retraction.[103] In New Zealand white rabbits that had undergone a closed tenotomy of the FDP tendon, numerous fine new vessels forming at the tip of lacerated tendons were shown. Adhesions that formed between tendons and the surrounding connective tissues were also highly vascularised.[104] Increased vascularity at the tendon stump following tendon division and suture was also shown in the flexor tendons of the canine forefoot.[29] Bidder *et al.* using *in situ* hybridisation in a canine model of tendon injury, identified cell populations within the repair site expressing message for VEGF, suggesting their potential for organising the angiogenic response during the

early postoperative phase of wound tendon healing.[105] Recently, Pufe *et al.* demonstrated that VEGF concentrations are negligible in healthy human adult Achilles tendons, but high in ruptured and embryonic ones. The splice forms detected in the area of rupture of torn Achilles tendons were VEGF121 and VEGF165. The same authors showed also that *in vitro* rat tenocytes stimulated with EGF raised VEGF secretion two to six fold, while hypoxic conditions alone (5% O2) raised VEGF secretion only twofold. However, the combination of EGF and hypoxia increased VEGF production 30- to 40-fold, apparently a synergistic effect.[106] Fenwick *et al* denounce the lack of research on the distribution of receptors for potential angiogenic GFs and receptors for those GFs that may be involved in tendon repair and advocate new studies in this field.[107]

Platelet-rich plasma

The first randomized controlled trial has been recently published in 2011 to assess the efficacy and safety of PRP augmentation for arthroscopic RC repair. The authors found no statistically significant difference between the two groups.[108]

In vivo animal models, growth factors seem to produce appropriate bone-to-tendon healing. It is unlikely that great changes will be observed through the application of individual growth factors. Because of this, there is much interest in the used of ''platelet-rich plasma'' (PRP) obtained by centrifuging autologous blood to purify a dense, suturable plasma matrix.[109]

PRP includes many of the growth factors identified previously as crucial in normal bone-to-tendon healing: TGF-b, bFGF, PDGF, VEGF, CTGF, and EGF.[110] *Although PRP has yet to prove itself as a biologic augmentation to rotator cuff tears, one human study has investigated the safety of PRP augmentation to rotator cuff repair.[109] A total of 14 patients undergoing arthroscopic repair of a rotator cuff tear received an intraoperative application of autologous PRP in combination with an autologous thrombin component after tear repair. The authors concluded application of PRP during arthroscopic rotator cuff repair is safe and effective, without any adverse events.*

In 2008, a case report was published by Maniscalco et al. [111] Anitua and colleagues [90] found faster recovery in athletes undergoing PRP enhanced Achilles tendon repair. In their study, athletes treated with surgery and PRP were compared with a retrospective control group of athletes treated with surgery alone. The PRP patients recovered range of motion earlier, had no wound complications, and returned to training activities in less time than control patients. The cross-sectional area of the PRP-treated tendons was also smaller than that in nontreated tendons when measured by ultrasound.

Recently, Volpi et al [112] described the local and systemic effects of ultrasound-guided autologous PRP injections in a variety of chronic tendinopathies in athletes between 17 and 68 years old. PRP injection produced symptom improvement well maintained for at least for two years from treatment. However, not all studies report a favorable effect of PRP [113], and caution should be exerted. [114, 115] *Further prospective studies are needed to investigate the effect of PRP on rotator cuff healing and ultimate shoulder function.*

Future perspectives

Growth factors can be delivered to the site of injury by direct application. This is the most straightforward method, and can be achieved via local injection, or by using impregnated sutures or scaffolds. Local injection is comparatively noninvasive, simple and quick. One disadvantage of local injection is the overflow loss. It can be avoided by using impregnated scaffolds [116] or sutures, which have the advantage of delivering the growth factor to the specific area of injury. In a study on RC sheep model suture have been coated with recombinant human platelet-derived growth factor BB (rhPDGF-BB). Histologic analysis showed improved tendon-to-bone healing in the rhPDGF-BB-augmented repairs.[117] However we should consider that rotator cuff repairs are commonly being done arthroscopically, and placing and securing the patch graft arthroscopically would be technically difficult for most surgeons. The main disadvantage of direct application is that growth factors only remain at the site for a short duration of time. As tendon healing continues for months

to years, this short duration of the presence of growth factors may not be effective enough. Nevertheless, several animal studies have demonstrated the beneficial results from local injection of growth factors.[118] Another factor to consider is the added cost of the commercial equipment used for these procedures. PRP includes many of the growth factors previously identified and the biological effects may depend on concentration of platelets.[119]

One technical problem using PRP 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. Than there are no clinical studies on the efficacy of PRP on rotator cuff healing and further prospective studies are needed to investigate its effect.

Many other problems remain unresolved for clinical use of GF. The best volume and frequency of the injections is unclear. When multiple injections are considered, the ideal period between multiple injections is unknown.[120] There has been little research performed on the amount of growth factors produced using different cell separating systems, and what the optimal mixture would be. It is also uncertain whether platelet activation prior to injection is necessary, as contact with collagen would also lead to platelet degranulation.[121]

Conclusions

The time course of GF expression is an important element in RC healing, and a better understanding of how, where, when and for how long such factors are expressed may help in the development of methods to manipulate their expression, accelerate healing, and reduce adhesions. The use of GFs in rotator cuff tears remains, for the time being, largely experimental.

Despite the lack of knowledge of the physiology of GFs expression and effects during the process of tendon healing, the therapeutic use of GFs for a wide variety of soft tissue ailments is empirically increasingly common. The study of the clinical effects of the GFs in humans is intrinsic to the idea of biological solutions to clinical problems, and is emerging as a new paradigm in medicine leading

to the development of novel and more optimized biological preparations which might open new avenues in surgery and the management of a wide range of conditions. From a clinical view point, despite the mounting laboratory evidence of the positive effects of GFs in tendon healing, only well conducted, appropriately powered randomised controlled trials, with adequate outcome measures and length of follow up will clarify whether GFs play a role in routine clinical practice.

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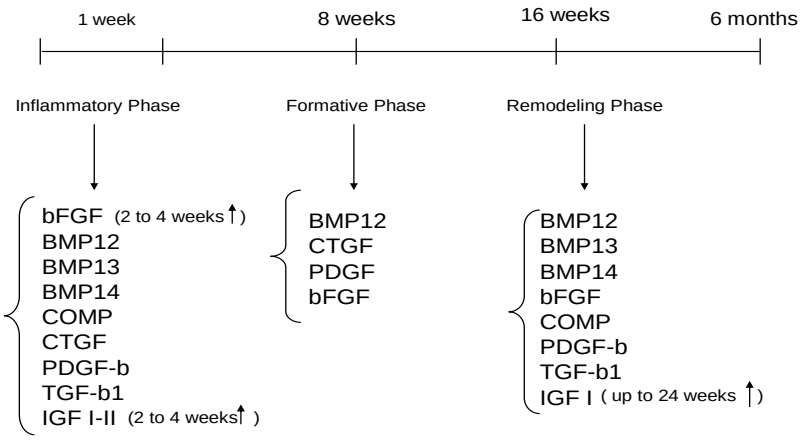
TABLE I



The healing process of all tendons passes through three well defined stages. Growth factors are expressed during all these phases, promoting cell proliferation, extracellular matrix production and also participating to the final remodeling of the tendon. This table shows the timing of some Growth factors studied. It is possible that, in the next future, it will be modified after new investigations, but it such timings must be kept in mind, because the addition of a given growth factor too early or too late in the tendon healing process may decrease their effectiveness.