Expert Opinion

1. Introduction
2. The early response to tendon injury
3. Platelet-rich therapies
4. Tendinopathy
5. Expert opinion

Tendon healing and platelet-rich plasma therapies

Isabel Andia[†], Mikel Sanchez & Nicola Maffulli
[†]*Osakidetza, Basque Health Service, Research, Zamudio, Spain*

Importance of the field: The therapeutic use of platelet-rich plasma (PRP) is an autologous biotechnology that relies on the local delivery of a wide range of growth factors and cytokines with the aim of enhancing tissue healing. Understanding both tendon healing and PRP therapies is an area of research that is critically important in developing optimal formulations and protocols to achieve the intended therapeutic effects.

Areas covered in this review: We summarise recent information on the mechanisms inherent to the earliest response to tendon injury. We then describe the positive effect of PRP therapies on tendon healing. Research on tendinopathy has produced several biological hypotheses based on histopathological, biochemical and clinical findings showing that cell apoptosis, angiofibroblastic features or abnormal biochemical adaptations underlie the condition.

What the reader will gain: The article provides insights into early healing mechanisms and the influence of PRP therapies on inflammation, cell migration, angiogenesis and the proliferation and synthesis of extracellular matrix. The knowledge gained helps to better understand and optimize tendon therapies.

Take home message: The use of endogenous therapies has a positive effect on experimental tendon healing. However, several obstacles need to be addressed to optimise medical practice in this field.

Keywords: cell signalling, healing, platelet-rich plasma, tendinopathy, tendon

Expert Opin. Biol. Ther. [Early Online]

1. Introduction

Tendon problems are a major cause of musculoskeletal morbidity. Indeed, an estimated 30 – 50% of all sports lesions are painful tendon injuries [1] that affect professional and recreational athletes in various anatomical locations. The location of these injuries is sport-specific. For example, Achilles tendinopathy is more prevalent in sports that have a large running component, while rotator cuff problems are frequent in sports requiring overhead motions and high-force throwing movements, and are prevalent in throwers and racquet sports players [2].

The Bone and Joint Decade (2001 – 2010) has witnessed the development of platelet-rich plasma (PRP) technologies to improve tissue repair, especially in the musculoskeletal system [3]. The rise of such therapies in tendon problems dictates that the present knowledge of both healing mechanisms and PRP therapies needs to be better explored to translate such knowledge into biological plausible therapies. Thus, we first summarize recent information on the mechanisms inherent to the earliest response to injury. We then describe the status of the field of PR therapies and question whether this knowledge can be applied for clinical benefit. Finally, we summarize the different hypotheses regarding the biological mechanisms underlying tendinopathy.

10.1517/14712598.2010.514603 © 2010 Informa UK, Ltd. ISSN 1471-2598

Article highlights.

- Tendon repair occurs through the dynamic and collaborative interactions of various cell phenotypes. However, extracellular and intracellular signaling is organized through intricate networks infinitely complex to elucidate.
- Achieving meaningful biological insights of the early healing response may drive the successful formulation and application of PRP therapies.
- PRP therapies are more complex than it first seemed, and the primacy of growth factors might be undermined by unveiling new classes of molecules.
- Although PRP therapies have many compositions and procedures for application, they all try and maximize the cell signals that may enhance tendon healing.
- Our increased understanding of the healing mechanisms that result in tendon repair and tendinopathy is paving the way towards the optimization of tendon therapies.

This box summarizes key points contained in the article.

2. The early response to tendon injury

The repair process occurs in tendons, as in other tissues, through a dynamic sequence of events characterized by a well ordered sequence of cell recruitment and signaling from various systems. When tendons are injured, each and every one of these cell lineages – immune, vascular and nervous cells as well as resident or migratory fibroblasts and tendon progenitor cells [4,5] – sense environmental changes and try to reverse the stressful alterations by initiating a healing cascade that results in complex biochemical changes (**Figure 1**). Current research aims to clarify the chronology of the different stages of early repair, which is probably defined by the temporal prevalence of particular cell types in addition to specific cellular signaling.

2.1 Neural response

After trauma, the initial stress energy is encoded into neural signals. Although the normal tendon is essentially devoid of neural components [6], ummyelinated axons that innervate the peritendon (adjacent loose connective tissue) and endotenon sense the molecular products of injury and transmit signals to modulate the efferent neural responses along with the immune responses [7]. In practice, much evidence regarding tendon healing points to the nervous system as the key to efficient repair, based on evidence such as improved healing after the application of neuropeptides such as calcitonin gene related peptide (CGRP) and substance P [8-10] or nerve growth factor (NGF) [11], and worse healing after denervation of the medial collateral ligament [12] or the Achilles tendon in rodents [13]. Certainly, denervated systems do not have the full range of physiological potential or the capability for integration of diverse responses that is required for physiological tissue healing. Likewise, the poor healing ability of articular cartilage may result from its aneural and avascular nature [14].

Nerves and vessels travel together, and a two-way relationship based on extensive cross-talk is required for proper tissue repair. For example, dynamic changes with spatial and temporal implications were shown in the healing of the rat Achilles tendon. During the proliferative phase, nerves (CGRP- and substance P-positive) and vessels from the peritendon sprouted within the injured tendon; thereafter, similar nerve fibers were identified in areas peripheral to the repair site, where they might help to reduce angiogenesis during remodeling [15].

2.2 Early inflammatory response

2.2.1 Participating immune cells

Acute inflammation, the complex systemic early defense system, is the first reaction of the innate immune system (platelets, leukocytes and macrophages) to injury. Direct exposure of cells to physical, mechanical or chemical trauma can have immunological consequences relative to the degree of injury, that is the apoptotic or necrotic condition of resident fibroblasts [16]. Accordingly, local regulatory mechanisms adjust the magnitude of the response so that inflammatory processes are localized to areas of damage and the amount and duration of immune cell infiltration are adequate to phagocytose apoptotic/necrotic cells. In addition, endothelial cells, which are actively involved in healing, limit clot formation to sites of injury. Activated platelets and leukocyte within this clot then release growth factors and numerous cytokines, establishing the onset of inflammation.

Eventually, spatially and temporally changing patterns of various leukocyte subsets transmigrate across the endothelium. Circulating neutrophils are rapidly captured by selectins and presented by endothelial cells; they then invade the tendon in response to chemical signals. The lifespan of neutrophils in the injured tissue is about two days, during which they perceive signals from the environment and respond by secreting cytokines [17]. Furthermore, neutrophils release stored substances carried in different granule subsets, including reactive oxygen species, cationic peptides or proteases.

2.2.2 Activation of macrophages

Monocyte recruitment and infiltration at the injury happens days later, and is highly regulated by adhesion molecules expressed by endothelial cells and by chemokines and other substances released by platelets, neutrophils [18] and apoptotic/necrotic cells [19]. Commanded by signals present in the environment, monocytes turn into macrophages (the dedicated phagocytes), inducing major changes in gene expression and cell function. Indeed, the severity of tissue injury may determine different states of macrophage activation, including 'innate' activation through lipolysaccharide or IFN-γ associated with a pro-inflammatory state (production of IL-6, IL-1β and TNF-α) [16] or 'classical' activation through IL-4/IL-23 associated with the synthesis of healing factors including transforming growth factors (TGF-β and -α), basic fibroblastic growth factor (bFGF), platelet-derived growth factor (PDGF) and VEGF [20].

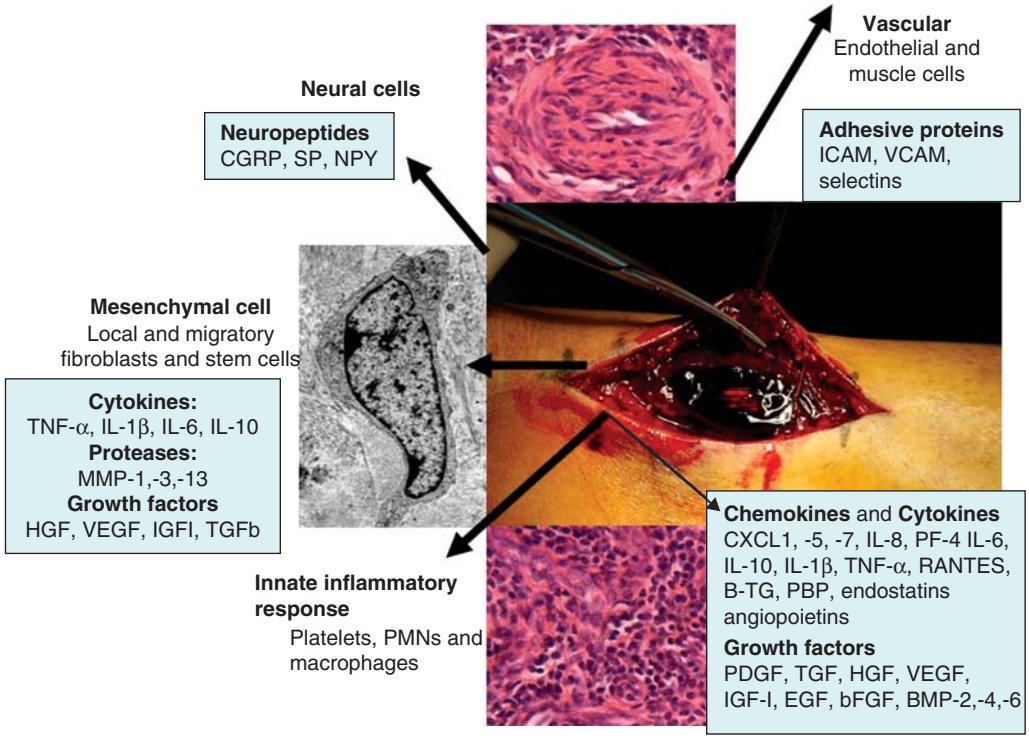

Figure 1. The early response to tendon injury. Considerable complexity is evident at all levels of organization from macromolecules and cells to tendon tissue. Such complexity entails participation of various cell lineages, including neural, vascular, innate inflammatory cells, stem cells and fibroblast-like cells (local and migratory). At the molecular level, intercellular signals (blue boxes) and their receptors control the molecular dialogue between these cells. Intracellular and extracellular signaling is organized through intricate networks infinitely complex to elucidate. Early repair comprises integrated, interactive stages whose properties as a whole exceed those of individual molecules, cells or stages.

bFGF: Basic fibroblastic growth factor; BMP: Bone morphogenetic protein; B-TG: Beta-Thromboglobulin; CGRP: Calcitonin-gene-related peptide; HGF: Hepatocyte growth factor; ICAM: Intercellular adhesion molecules; NPY: Neuropeptide Y; PBP: Platelet basic protein; PDGF: Platelet-derived growth factor; PF-4: Platelet factor-4; PMNs: Polymorphonuclear leukocytes; RANTES: Regulated on activation normal T cell expressed and secreted; SP: Substance P; VCAM: Vascular cell adhesion molecule.

2.2.3 Critical role of immune mediators

Recent research suggests that these features of inflammation may determine the choice between efficient repair or failure to repair. For example, in animal experiments, neutropenia accelerated the closure of incisional wounds [21], but did not affect the healing of surgically repaired tendons [22]. Macrophage depletion impaired skin wounding by reducing collagen deposition and angiogenesis [23], and also impaired the response to wounding in diabetic mice [24]. Other studies suggest that targeting macrophage activation may provide a new therapeutic approach to protect tissues from ischemia and promote repair [25]. Notwithstanding, macrophage depletion significantly improved the morphology and biomechanical properties of the tendon–bone interface after experimental anterior cruciate ligament (ACL) surgery [26]. Thus, there are large gaps in understanding how neutrophils and macrophages influence repair. The problems with understanding the inflammatory response stem, in part, from its biological redundancy: by which one molecule may have several functional roles, and different molecules may perform overlapping functions [27].

2.3 Cell proliferation and angiogenesis

The high concentration of growth factors and cytokines initially secreted by platelets and leukocytes and later amplified by macrophages induce a rapid increase in specific cell populations, including endothelial cells, migrating fibroblasts and resident tendon cells. That the number of tenocytes increases in parallel with angiogenesis is readily evident in the hypoxic environment produced by the tendon injury.

Animal studies have shed some light on the natural pattern of growth factor expression during this stage. For example, signaling of VEGF-A via the endothelial receptors VEGFR1 and VEGFR2 is present at the healing site early after tendon injury [28]. Other growth factors, including TGF-β, PDGF-BB, and angiopoietin-1, which are important for vessel stabilization, are expressed later at the healing site [29].

Growth factors, including TGF-β1 PDGF, brain-derived neutotrphic factor (BDNF), bFGF and IGF-I, function at various stages during the healing process, producing different outcomes depending on the conditions. For example, early mobilization post-rupture up-regulates the expression of

several growth factors (BDNF, bFGF, COX 1 and hypoxia-inducible factor (HIF)-1 alpha) and may accelerate healing [30]. In the same way, cells use their receptors in an energy-efficient and flexible manner. For instance, PDGF, a chemotactic and mitotic factor for fibroblasts, also induces the synthesis of collagen type I. TGF-β1, which peaks early in tendon injuries [29], is essential for the recruitment and maintenance of tendon progenitor cells during tendon formation [31], and its function might be necessary during tendon healing. Additionally, the interactions of TGF-β1 with other TGF-β isoforms, namely -2 and -3, mediate which type of collagen is synthesized in the healing tendon [32]. IGF-I anabolic and antiapoptotic activities are regulated by IGF-I binding proteins -2, -3, and -4, which are also present in the early healing response [33]. In both humans and animals [34], the expression of IGF-I and TGF-β1 precedes the stimulation of collagen synthesis, a relevant issue in tendon healing [35].

During every phase (i.e., inflammation, angiogenesis and proliferation), the specific signaling activity is in a fine balance with other endogenous signals that serve to limit the duration of the phase and progress to a new phase.

3. Platelet-rich therapies

Generally speaking, we use the term 'PRP therapies, to include all platelet-rich plasma, technologies and re-administration procedures to patients.

3.1 How may PRP therapies act upon early tendon healing?

Platelets, which are produced by megakaryocytes in the bone marrow, comprise up to $1.4 - 4 \times 10^{11}$ cells per litre of blood and circulate for about 10 days. To exert their healing function, platelets become activated at the site of tissue injury. Adhesion and activation, along with fibrin formation, then cause the release of intracellular stores – predominantly α-granules (50 – 80 α-granules per platelet), dense granules (3 – 5 granules per platelet) and lysosomes [36]. To date, the effectors of the beneficial function of PRP therapies are growth factors such as PDGF, TGF, FGF, endothelial growth factor (EGF), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF) and VEGF, among others. However, this process seems to be more complex because α-granules contain more than 300 proteins [37]; thus, the primacy of growth factors might be undermined by unveiling whole new classes of molecules. In addition to platelets and plasma-signaling proteins, PRP therapies contain structural proteins (e.g., fibrin, fibronectin and vitronectin) which facilitate cell adhesion by forming three-dimensional scaffolds.

3.1.1 *Inflammation*

Platelets modulate inflammation largely because of their ability to secrete high levels of chemokines (a subset of small, diffusible cytokines), required to control trafficking and the further accumulation of leukocytes and monocytes in the injured tissue [38]. Supporting this hypothesis, the inhibition of platelet activation with antiplatelet glycoprotein Ib (GP-Ib) decreased polymorphonuclear leukocytes influx by 50% [39]. Indeed, platelets augment the local concentration of relevant chemokines, including CXCL1/GRO, ENA78/CXCL5, monocyte chemoattractant protein-1 (MCP-1), CCL5 and IL8 [40]. In addition, platelets are considered the major source of β-thromboglobulin [(CXCL7 or neutrophil activating peptide-2, (NAP-2)], a strong chemoattractant and an activator for neutrophils. Platelets secrete two CXCL7 precursors (i.e., platelet basic protein (PBP) and connective tissue activating peptide-III (CTAP-III)), but proteolytic processing by neutrophils is essential to make chemotactically active CXCL7. Hence, we may infer that leukocyte–platelet-rich fibrin will attract more neutrophils from the blood stream than platelet-rich fibrin alone. This issue, although needing experimental confirmation, may be clinically relevant when deciding to use pure platelet-rich plasmas (PRPs) or leukocyteplatelet concentrates. *A priori*, we exclude neutrophils because they may exacerbate tissue damage via several different mechanisms (i.e., secreting pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6 or IL-1β) that cause matrix destruction through the production of MMP-1, -3 and -13 [41]. Furthermore, TNF-α strongly activated the tenocytes and amplified TNF-α production, thus upregulating the expression of MMP-1 and pro-inflammatory IL-1β, IL-6 and IL-10 cytokines [42]. In addition, the interaction of neutrophils with platelets may induce a hyperactive leukotactic response of circulating neutrophils toward the injury site. Thus a large influx of these cells into the injury and subsequent activation of oxidative and enzymatic processes can intensify host tissue damage.

Further basic sciences knowledge could help in refining PRP therapies. For example, inducing 'classical' macrophage activation while avoiding 'innate' activation may produce an anti-inflammatory environment. Indeed, preliminary studies indicate a high level of complexity in macrophage activation states dependent on the nature of the stimulants or the combination of stimulants to which they are exposed [43]. Minimal research has been conducted to investigate PRP formulation at this level, and limited preliminary experiments [44] have shown an inflammatory suppression in the short-term followed by an increase of the inflammatory cytokine IL-1β.

Tendon fibroblasts, the crucial target cell for PRP therapies, contribute to the resolution of inflammation by normalizing chemokine gradients, thereby allowing infiltrating leukocytes to undergo apoptosis or to leave the tissue through the draining lymphatics.

3.1.2 *Stem cell and fibroblast migration*

Representing 25% of the content of alpha-granules, platelets are the major source of platelet factor-4 [45], which, in cooperation with PDGF and CXCL7, activates fibroblasts' migration [46]. Platelets also provide initial cues, such as PDGF-B, bFGF and CXCL5, for the homing of precursor cells to the

injury [47]. Accordingly, Kajikawa *et al.* reported the importance of preserving the blood supply in the injured tissue; they found an increase in circulation-derived cells in tendons treated with injections of PRP [48].

3.1.3 Angiogenesis

Platelets support angiogenesis, but these molecular mechanisms are just beginning to be understood. Platelet α-granules contain a variety of both pro- and anti-angiogenic proteins; the former include growth factors such as platelet-derived growth factors (PDGF AB or C), TGF-β1, VEGF, HGF and other soluble cytokines (namely, chemokines IL-8, angiopoietin CXCL12 and MMPs -1, -2 and -9 [49]. These angiogenic activators collectively promote vessel wall permeability and recruitment and the growth and proliferation of vascular cells.

Paradoxically, α-granules also contain established inhibitors of angiogenesis such as thrombospondin-1 (TSP-1), modulating vascular cell behavior by altering endothelial and vascular smooth muscle cell adhesion, proliferation, motility and survival [50]. For example, PRP containing a fourfold concentration of platelets releases 183 ± 21 μg of TSP-1/ml [51]. By preventing VEGF and bFGF binding, TSP-1 interferes with their mitogenic effects; in addition, it inhibits nitric oxide signaling [50]. Other anti-angiogenic proteins in PRPs are angiostatin, endostatin and fibronectin and the tissue inhibitors of metalloproteinases (TIMPs -1 to -4) [49]. There is some evidence that pro- and anti-angiogenic proteins may be stored separately and differentially released because the secretion of pro- versus anti-angiogenic stores may be agonist-specific [52].

Furthermore, vesicles within platelets (i.e., dense granules) store and deliver a pool of small molecules such as histamine, noradrenaline, dopamine and serotonin, which increase vascular permeability by allowing the extravasation of plasma proteins into the injury, an event diminished by 25% if platelet secretion is impeded through anti-platelet GPIb pre-treatment [39].

3.1.4 Cell proliferation

Rapidly achieving a high number of resident and precursor cells is important because the synthesis of matrix proteins rises proportionately. Human tenocytes treated with PRP increased their proliferation and were stimulated to release VEGF and HGF [53,54]. The cooperative paracrine actions of these factors will promote angiogenesis, which is directly related to the tendon-healing capability. VEGF also increases vascular permeability [55]. Additionally, as HGF is a potent antifibrotic, it may help to reduce scar formation around tendon tissues. Further research in a sheep model showed that repetitive injection of activated PRP within Achilles tendon fascicles triggered a healing response, as assessed by increased cell number and angiogenesis, and did not provoke fibrosis [56]. Accordingly, Lyras *et al.* [57] found a higher degree of neovascularization and faster remodeling in the patellar tendons and in the Achilles tendons of New Zealand rabbits treated with PRP. Also, injections of PRP one week post-operatively increased tendon regeneration and strength [58]. PRP primarily stimulates tendon cells to produce high levels of hyaluronan and type I collagen [59]. Other platelets and plasma proteins are both required to stimulate collagen gene expression [60]. Recently, a placebo-controlled experimental study showed improved mechanical properties, higher strength at failure and elastic modulus coupled with increased metabolic activity, in horse superficial digital flexor tendons treated with PRP [61]. Combining these experimental findings and translating them into clinical investigation was the next logical step.

3.2 Summary of clinical data

The feasibility and biosafety of PRP therapies made possible their application in both surgical and conservative management of tendon problems. Preliminary clinical evidence was modest given the methodological limitations and difficulties in comparisons caused by the heterogeneity in the plasma products and the different protocols for application used in the studies. Initially, observational studies reported significant functional improvement on arthroscopic rotator cuff repair and refractory jumper's knee [62,63]. Likewise, Everts *et al.* [64] reported better functional recovery and less pain in open subacromial decompression in a prospective, randomized double-blind study. Furthermore, improved functional recovery after surgical management of Achilles tendon tears was achieved by using a well-defined PRP technology. The effect induced by PRP therapies had long-term consequences such as decreased cross-sectional area after 18 months [65].

Conservative management with PRP injections is becoming more widespread. Recently, three studies on PRP injection, of which two were on patients with chronic patellar tendinopathy [63,66] receiving three injections of leukocyte-platelet concentrate (double centrifugation), were reported. Significant improvements in Tegner scores were described in one of the two studies. In addition, improvement in pain and function was reported after a single PRP injection in patients with epycondylitis [67]. More recently, two double-blind, randomized clinical trials were performed, one on patients with lateral epycondylitis [68] and the other on patients with chronic Achilles tendinopathy [69], respectively; in both studies, the experimental treatment consisted of a single injection of an identical buffered PRP. The clinical results were significant for patients with tennis elbow, for which PRP reduced pain and improved function. However, the control group had received corticosteroid injections known to produce worse long-term outcomes than a wait-and-see policy [70]. In patients with Achilles tendinopathy, PRP injection did not improve pain reduction and activity [69]. No complications were reported after PRP treatments, but the evidence for or against the beneficial effect of PRP therapy in chronic tendon problems needs to be clarified.

3.2.1 Critical parameters: composition, activation and administration procedure

Fundamental differences between PRP products and applications might have important implications for clinical

outcomes, raising controversial opinions regarding their therapeutic value and calling for comparative effectiveness research. The success of PRP therapies depends on clarifying the optimal procedure for administration, the characteristics of the plasma and its activation and the volume used. However, this issue is challenging because PRP therapies influence the output of multiple mechanisms and also because we are far from a quantitative understanding. Optimal formulations and protocols that take individual conditions into account are needed; for example, our clinical experience involves PRP prepared through single-step centrifugation containing two to three times the normal platelet concentration and no leukocytes. In contrast, PRPs obtained through double-centrifugation or filtration produce platelet-leukocyte concentrates four to six times those found in peripheral blood. Some protocols do not activate plasma because contact with collagen would activate clotting [71]. Instead, we activate plasma with calcium chloride prior to injection; this process results in fibrin confinement and proteins secreted gradually from the matrix (**Figure 2**). Usually, these proteins have to accumulate over time to reach the threshold set by the affinity of their receptors, a physiological mechanism found in many cellular processes *in vivo* [72]. To reach these thresholds, further cells synthesizing additional amounts of growth factors and cytokines are thus crucial. Hence, we also inject 'healthy' peripheral tissue targeting fibroblasts.

The optimal volume and number of injections is still unclear. It seems logical that these should be tailored to each patient taking into account the severity and location of the injury and the clinical response. High volumes have further mechanical advantages [73]. When multiple injections are considered, the ideal period between injections is unknown. However, because PRP therapies influence early healing, one week may be adequate for monitoring individual outcomes and making decisions about further plasma injections. In general we perform two or three PRP injections weekly on an outpatient basis. Ultrasonographic monitoring drives our clinical decision as to whether to perform additional injections or not. Because the procedure is not very painful we do not administer anaesthesia, instead ice is applied for about ten minutes after the plasma injection.

4. Tendinopathy

The term tendinopathy is used to describe the clinical picture of pain and swelling of a tendon associated with the histopathological findings of intratendinous failed healing response and no classical signs of inflammation [74]. These conditions may occur when tissue breakdown exceeds the rate of tissue healing or when the capacity for tissue repair is impaired. Thus, current hypotheses on pathophysiology focus on cell apoptosis, deregulated angiogenesis or pain and inflammation (**Figure 3**) [75]. These hypotheses are not mutually exclusive, because these processes may occur simultaneously or correspond to different temporal stages.

4.1 Cell apoptosis

Tendon homeostasis requires a fine balance between cell proliferation and cell death. However, intrinsic or extrinsic factors may cause imbalance resulting in excessive cell loss and tendon tissue disruption. One of the extrinsic causes of apoptosis may be a relative hypovascularity in the mid-portion of the Achilles tendon or supraspinatus [75]. In these hypovascular areas, exercise-induced hyperthermia can also result in cell death because the ability to regulate temperature is hampered. Another trigger for extrinsic apoptosis may be the loss of homeostatic tension from microscopic collagen breakdown during demanding exercise or overuse [76]. Indeed, tendon cells lacking appropriate extracellular matrix (ECM) attachment are rapidly eliminated by means of apoptosis.

Alternatively, the intrinsic pathway of apoptosis (Bcl-2-inhibitable or mitochondrial) functions in response to various types of intracellular stresses including DNA damage, unfolding stress in the endoplasmic reticulum and death-receptor stimulation. Early apoptotic cells can release chemotactic factors to attract phagocytes towards sites of apoptotic cell death. The induction of apoptosis also elicits the release of lactoferrin, an anti-inflammatory glucoprotein that selectively inhibits the migration of neutrophils towards early apoptotic cells, preventing any aberrant inflammation at sites of physiological cell death.

At least one study has shown a large number of apoptotic cells in ruptured supraspinatus tendons [77], and other studies showed excessive apoptosis in tendinopathic patellar tendon specimens in athletes [78] and non-insertional Achilles tendinopathy [79]. More recently, additional molecular aspects of the tendon apoptotic process were elucidated in rat studies. Using a microarray approach, Millar *et al.* [80] reported downregulation of anti-apoptotic heat shock proteins (HSP27 and HSP70) and upregulation of pro-apoptotic genes such as caspase-3 and -8 and FADD-like IL-1β converting enzyme (FLICE) inhibitory protein; importantly, all these findings were confirmed at the protein level in degenerative human supraspinatus tissues.

Agents capable of counteracting apoptotic changes are not clinically available, but some preclinical research has been reported. For example, IGF-I, which is present in plasma at a concentration of 50 – 100 ng/ml, prevented anoxic apoptosis in Achilles tendon cells [81]. Cartilage oligomeric protein (COMP), also present in plasma, protects cells against death by elevating members of the inhibitors of apoptosis (IAP) family of survival proteins [82]. Thus, exploring the anti-apoptotic potential of plasma preparations is another logical step to advance our knowledge in this field.

4.2 Deregulated angiogenesis

A typical histopathological feature in tendinopathy is prominent fibroblastic cellularity and haphazard neovascularization, suggesting that a failed healing response may lead to a chronic state of non-finalized angiogenesis [83,84]. The accumulation of blood vessels is consistent with overproduction of vascular

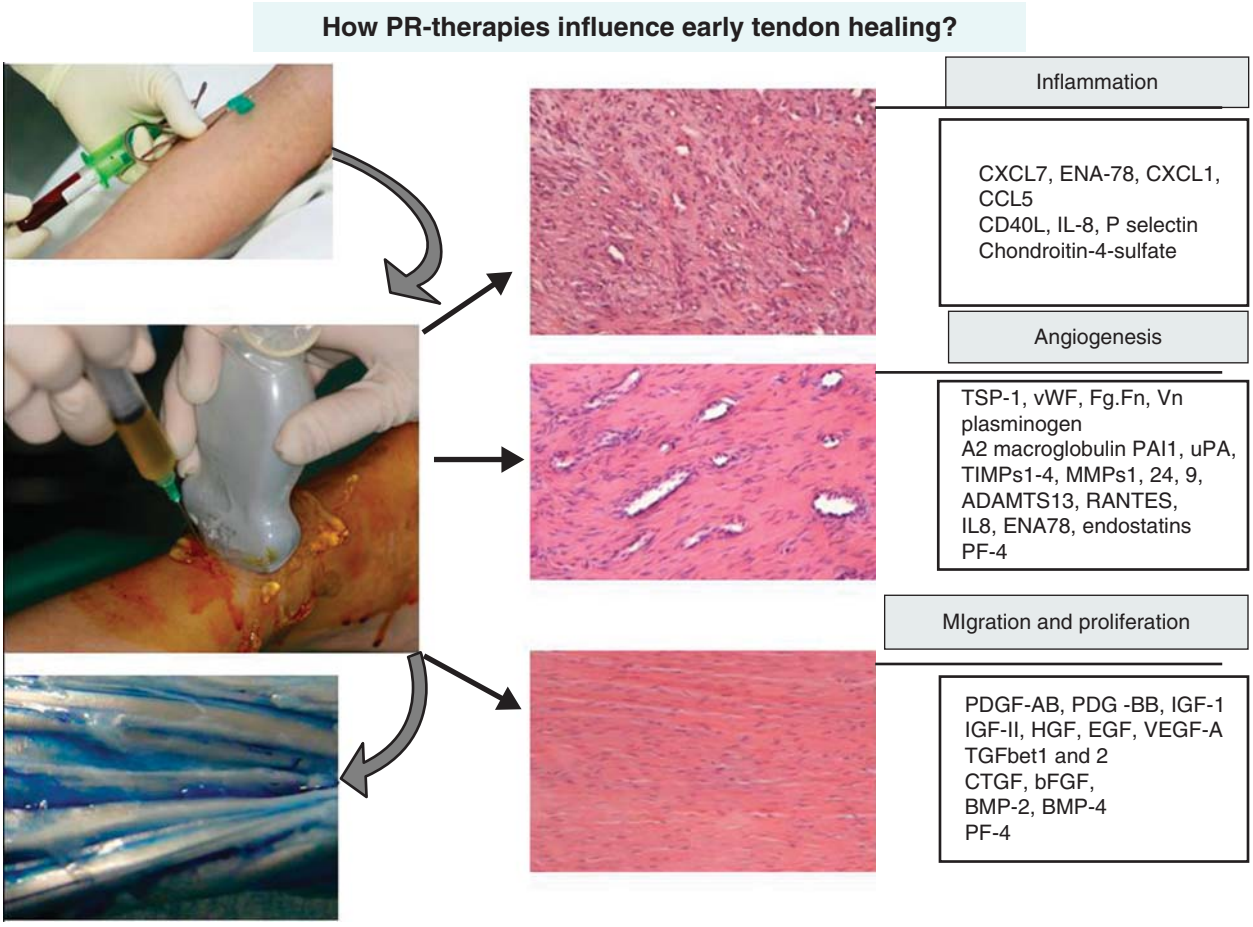

Figure 2. Insights into how PRP-therapies influence early healing mechanisms. PRP therapies, prepared from autologous blood, consist of 3D fibrin matrices, which function as platforms for cell adhesion; furthermore, these matrices hold activated platelets (optionally leukocytes) that gradually secrete signaling factors (boxed) in the injury milieu. In doing so, they modify inflammation, angiogenesis and also cell migration, proliferation and the synthesis of extracellular matrix proteins (not shown). These changes may contribute to enhanced tendon healing. However, the success of PRP therapies depends on clarification of the optimal plasma formulation and procedure of administration, the characteristics of the plasma, its activation, and the volume used. In the percutaneous injection, the goal is to control the activation degree of the preparation. Therefore, we inject the plasma shortly after $CaCl_2$ addition within the focus of altered tendon substance and adjacent areas. The injected biomaterial did not wash away since a platelet-rich fibrin develops within the target site, releasing growth factors into the pathological and adjacent areas such as the pre-tendinous fat and the space between the tendon and the paratenon aiming to impact tenoblasts and mesenchymal cells from these areas. At the left-bottom, we show ink blue-stained plasma clotted within tendon fascicles after our preferred mode of administration.

ADAMTS: A disintegrin-like and metalloprotease domain with a thrombospondin motif; bFGF: Basic fibroblastic growth factor; BMP: Bone morphogenetic protein; CTGF: Connective tissue growth factor; ENA78: Epithelial cell-derived neutrophil-activating peptide 78; Fg: Fibrinogen; Fn: Fibronectin; HGF: Hepatocyte growth factor; PAI1: Plasminogen activator inhibitor; PDGF: Platelet-derived growth factor; PF-4: Platelet factor-4; RANTES: Regulated on activation normal T cell expressed and secreted; TIMP: Tissue inhibitor of metalloproteinases; TSP: Thrombospondin; Vn: Vitronectin; vWF: von Willebrand factor.

factors, particularly VEGF [85]. VEGF is crucial for neovessel growth, but it does not participate in neovessel stabilization; hence, tendinopathic patients may demonstrate angiofibroblastic features without VEGF expression [85]. Observational data link high blood flow and prominent perivascular sympathetic innervation in the paratenon with painful tendinopathy in both the patellar and Achilles tendon [86,87]. In these conditions, clinical experience shows that sclerosing injections targeting the point where the blood supply enters the tendon reduce pain – probably by interfering with the local nerve supply [88]. Another therapy directed at modifying blood flow uses glyceril trinitrate patches, which deliver NO transcutaneously. NO relaxes vascular smooth muscle cells, increases blood vessel diameter and flow and is singularly coupled with pain reduction. Three clinical trials involving different anatomical locations (tennis elbow, Achilles and supraspinatus) showed that NO delivery via a patch reduced pain and increased tendon strength and mobility [89].

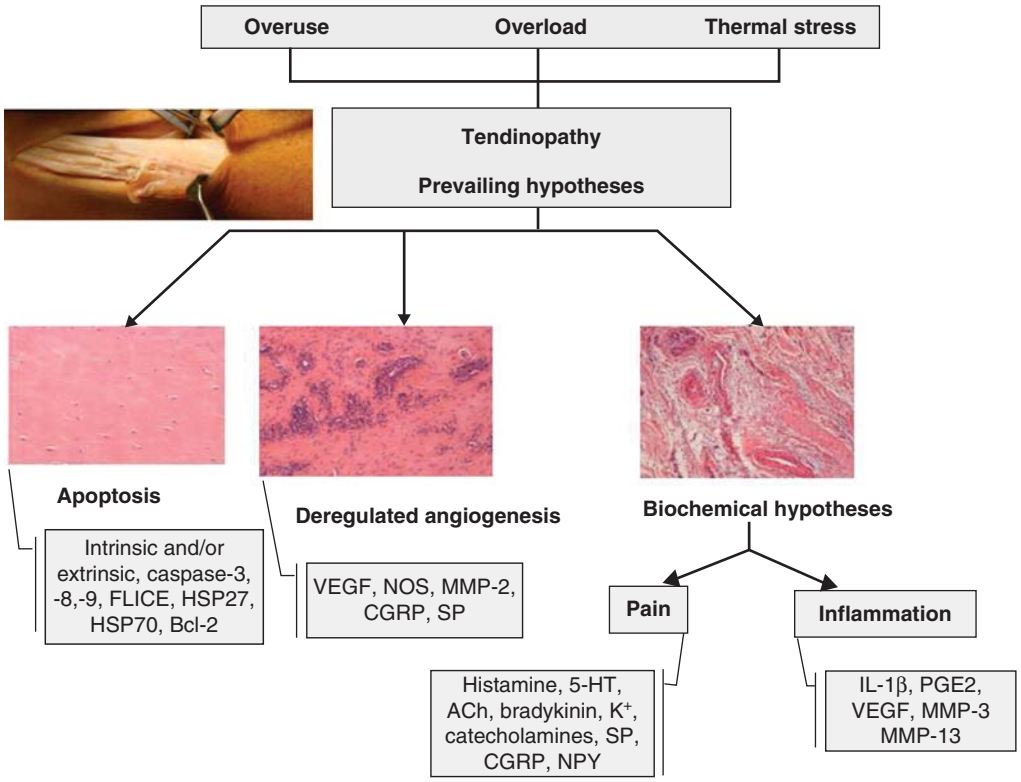

Figure 3. Prevailing biological hypotheses of tendinopathy. Repetitive mechanical, chemical or physical stresses followed by an abnormal healing response bring forth the failed healing response changes typical of tendinopathy. Prevailing biological hypotheses are based on various histopathological, biochemical and clinical findings showing cell apoptosis, angiofibroblastic features or an abnormal biochemical adaptation underlying inflammation and pain. Biological hypotheses are associated with molecular changes (the blue boxes include some representative molecules).

5-HT: Serotonin; ACh: Acetylcholine; Bcl-2: B cell lymphoma/leukemia 2; CGRP: Calcitonin-gene-related peptide; FLICE: FADD-like IL-1β converting enzyme; NPY: Neuropeptide Y; PGE2: Protaglandin E2; SP: Substance P.

4.3 Biochemical hypothesis

4.3.1 Inflammation

Although histological evidence of leukocyte infiltrates is scarce, there is evidence of an inflammatory environment within tendinopathy tendons. The biochemical adaptation of prolonged repetitive mechanical loading is characterized by the production of proinflammatory agents (e.g., cytokines such as IL-1β and TNF-α, protaglandins such as PGE2, and neuropeptides such as substance P and CTGF [90,91]). Moreover, mast cells were increased in the patellar tendons of patients with pain and swelling [92]. Similarly, cells harvested from patients with patellar tendinopathy expressed higher levels of PGE2 and COX-2 than controls. Furthermore, PGE2 induced the differentiation of tendon stem cells into adipocytes and osteocytes *in vitro* [93]. Some of the detrimental effects of inflammatory cytokines include upregulated VEGF production and enhanced production of MMP-1, MMP-3 and MMP-13 [41,94], both of which cause matrix destruction. However, ultrasound-guided peritendinous injections of Adalimumab (a TNF blocker) or Anakinra (an IL blocker) in Achilles tendinopathy had only a modest clinical effect in a small trial [95].

4.3.2 Pain

Biochemical mediators in tendons might influence or irritate nociceptors in and around the tendon [96]. Indeed, sensory nerve fibers from the peritenon sprout within the chronic painful tendons and mimic the proliferative phase [86]. Some of the chemical substances known to modulate pain are histamine, bradykinin, serotonin, glutamate, acetylcholine (ACh), extracellular K^+, BDNF and catecholamines. Some of these agents, including catecholamines, ACh [97], transporters VAChT and ChT [98], BDNF, NGF [99] and glutamate are produced by transformed tenocytes within the degenerative tendon [100]. Glutamate is an excitatory neurotransmitter in the CNS, and is also present in tendons, where it may function as a regulatory cytokine. The involvement of glutamate in pain sensitization has been reported [101], and NMDA receptors are reportedly involved in glutamate-induced pain. As measured by microdialysis and immunohistochemistry, higher concentrations of glutamate are present in patients with Achilles and patellar tendinopathy, and tennis elbow [102] at levels high enough to modulate pain [103]. Accordingly, transformed tenocytes contained the vesicular glutamate transporter VGluT2 necessary

for glutamate secretion and NMDA receptors [104]. These and other results emphasized the importance of peripheral glutamate receptor antagonists in pain management for tendinopathy.

5. Expert opinion

Achieving rapid pain relief and tendon healing and developing conservative treatments to manage tendinopathy remain important objectives in the care of athletic patients who are involved in sports. However, achieving control of healing mechanisms is difficult, and the challenges associated with tendon healing and PRP therapies are enormous, extending beyond our present knowledge. The optimal platelet-rich plasma formulation and the protocol for treating patients with tendon injuries and pathologies is unknown, and may involve different formulations at different stages of healing or for patients with different histopathological or biochemical features. Further studies to determine the early mechanisms and the different temporal stages of tendinopathy will help to classify patients and monitor the progression of tendinopathy, and should be a high priority for researchers.

There is great potential for improvement by taking research findings and translating them into clinical investigation. Because of the safety of these products, basic science, clinical discovery and patient-oriented research should be inter-dependent and not successive steps. To achieve clinical benefits, we may be able to exploit future studies on macrophage activation and tendon cell apoptosis and to fine-tune neural responses and angiogenesis. The fundamental differences between PRP technologies should be identified and comparative effectiveness research should be performed. With good experimental data and extensive clinical details in hand, we hope to find optimal formulations and protocols to achieve the desired therapeutic effect. Of course, combining biological advancements with the art of medicine (i.e., knowledge, intuition and judgment) will be central for the proper translation of these therapies and their implementation in routine care.

Acknowledgements

We would like to thank Paco Angulo from the Athletic Bilbao football club for helpful discussion.

Declaration of interests:

The authors state no conflict of interest and have received no payment for the preparation of this manuscript.

Bibliography

1. Kannus P, Natri A. Etiology and pathophysiology of tendon ruptures in sports. Scand J Med Sci Sports 1997;7:107-12
2. Paalova M, Kannus P, Jarvinen M. Epidemiology of tendon problems in sports. In: Maffulli N, Renstrom P, Leadbetter W, editors, Tendon injuries: basic science and clinical medicine. Springer, London; 2005
3. Sanchez M, Anitua E, Orive G, et al. Platelet-rich therapies in the treatment of orthopaedic sports injuries. Sports Med 2009;39:345-54
4. Bi Y, Eirchiou D, Kilts TM, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 2007;13:1219-27
5. Tempfer H, Wagner A, Gehwolf R, et al. Perivascular cells of the supraspinatus tendon express both tendon and stem cell related markers. Histochem Cell Biol 2009;131:733-41
6. Ackermann PW, Salo PT, Hart DA. Neuronal pathways in tendon healing. Front Biosci 2009;14:5165-87
7. Tracey KJ. Reflex control of immunity. Nat Rev Immunol 2009;9:418-28
8. Stayaert AE, Burssens PJ, Vercruysse CW, et al. The effects of substance P on the biomechanic properties of ruptured rat Achilles' tendon. Arch Phys Med Rehabil 2006;87:254-8
9. Burssens P, Steyaert A, Forsyth R, et al. Exogenously administered substance P and neutral endopeptidase inhibitors stimulate fibroblasts proliferation, angiogenesis, and collagen organization during Achilles tendon healing. Foot Ankle Int 2005;26:832-9
10. Carlsson O, Schizas N, Li J, Ackermann PW. Substance P injections enhance tissue proliferation and regulate sensory nerve ingrowth in rat tendon repair. Scand J Med Sci Sports 2010: published online 10 March 2010, doi:10.1111/j.1600-0838.2009.01080.x
11. Mammoto T, Seerattan RA, Paulson KD, et al. Nerve growth factor improves ligament healing. J Orthop Res 2008;26:957-64
12. Ivie TJ, Bray RC, Salo PT. Denervation impairs healing of the rabbit medial collateral ligament. J Orthop Res 2002;20:990-5
13. Yeung CK, Guo X, Ng GYF. Denervation impairs Achilles tendon healing in a rat model. 2005 ISB XXth Congress – ASB 29th Annual Meeting; 805. Available from: http://www.asbweb.org/conferences/2005/pdf/0805.pdf
14. Nelson L, Fairclough J, Archer CW. Use of stem cells in the biological repair of articular cartilage. Expert Opin Biol Ther 2010;10:43-55
15. Ackermann PW, Ahmed M, Kreicbergs A. Early nerve regeneration after Achilles tendon rupture – a prerequitie for healing? A study in the rat. J Ortho Res 2002;20:849-56
16. Poon IK, Hulett MD, Parish CR. Molecular mechanisms of late apoptotic/necrotic cell clearance. Cell Death Differ 2010;17:381-97
17. Borregaard N, Sorensen OE, Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. Trends Immunol 2007;28:340-5
18. Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release granule proteins. Thromb Haemost 2009;102:198-205

19. Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol 2006;6:173-82

20. Krysko DV, D'Herde K, Vandenabeele P. Clearance of apoptotic and necrotic cells and its immunological consequences. Apoptosis 2006;11:1709-26

21. Dovi JV, DiPietro LA. Accelerated wound closure in neutrophil depleted mice. J Leukoc Biol 2003;7:448-55

22. Godbout C, Bilodeau R, Van Rooijen N, et al. Transient neutropenia increases macrophage accumulation and cell proliferation but does not improve repair following intratendinous rupture of Achilles tendon. J Orthop Res 2010;28:1084-91

23. Mirza R, DiPietro LA, Koh TJ. Selective and specific macrophage ablation is detrimental to wound healing in mice. Am J Pathol 2009;175:2454-62

24. Khanna A, Biswass S, Shang Y, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One 2010;5:e9539

25. Brechot N, Gomez E, Bignon M, et al. Modulation of macrophage activation state protects tissue from necrosis during critical limb ischemia in thrombospondin-1 deficient mice. PLoS One 2008;3:e3950

26. Hays PL, Kawamura S, Deng XH, et al. The role of macrophages in early healing of a tendon graft in a bone tunnel. J Bone Joint Surg Am 2008;90:565-79

27. Sercarz EE, Maveracki E. Recognition and function in a degenerate immune system. Mol Immunol 2004;16:737-45

28. Tsubone T, Moran SL, Amadio PC, et al. Expression of growth factors in canine flexor tendon after laceration in vivo. Ann Plast Surg 2004;53:393-7

29. Chen CH, Cao Y, Wu YF, et al. Tendon healing in vivo: gene expression and production of multiple growth factors in early tendon healing period. J Hand Surg 2008;33A:1834-42

30. Bring D, Reno C, Remstrom P, et al. Prolonged immobilization compromises up-regulation of repair genes after tendon rupture in a rat model. Scand J Med Sci Sports 2010;20:411-17

31. Pryce BA, Watson SS, Murchison ND, et al. Recruitment and maintenance of tendon progenitors by TGFbeta signalling are essential for tendon formation. Development 2009;136:1351-61

32. Chan K-M, Fu S-Ch, Wong Y-P, et al. Expression of transforming growth factor beta isoforms and their roles in tendon healing. Wound Rep Reg 2008;16:399-407

33. Dahlgren LA, Mohammed HO, Nixon AJ. Expression of insulin-like growth factor binding proteins in healing tendon lesions. J Orthop Res 2006;24:183-92

34. Kjaer M, Landberg H, Heinemeir K, et al. From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scand J Med Sci Sports 2009;19:500-10

35. Riley G. Tendinopathy-from basic science to treatment. Nat Clin Pract Rheumatol 2008;4:82-8

36. Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev 2009;23:177-89

37. Maynard DM, Heijnen HFG, Horme MK, et al. Proteomic analysis of platelet alpha-granules using mass spectrometry. J Thromb Haemost 2007;5:1945-55

38. Semple JW, Freedman J. Platelets and innate immunity. Cell Mol Life Sci 2010;67:499-511

39. Min-Ho K, Fitz-Roy EC, Scott IS. Dynamics of neutrophil extravasation and vascular permeability are uncoupled during aseptic cutaneous wounding. Am J Physiol Cell Physiol 2009;296:848-56

40. Flad H-D, Brandt E. Platelet-derived chemokines: pathophysiology and therapeutic aspects. Cell Mol Life Sci 2010;67:2363-86

41. Anitua E, Sanchez M, Nurden AT, et al. Platelet released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. Rheumatology 2007;46:1769-72

42. John T, Lodka D, Kohl B, et al. Effect of pro-inflammatory and immunoregulatory cytokines on human tenocytes. J Orthop Res 2010;28:1071-7

43. Menzies FM, Henriquez FL, Alexander J, Roberts CW. Sequential expression of macrophage anti-microbial/inflammatory and wound healing markers following innate, alternative and classical activation. Clin Exp Immunol 2010;160:369-79

44. Woodall J, Tucci M, Mishra A, et al. Cellular effects of platelet-rich plasma interleukin 1 release from PRP treated macrophages. Biomed Sci Instrum 2008;44:489-94

45. von Hundelshausen P, Petersen F, Brandt E. Platelet derived chemokines in vascular biology. Thromb Haemost 2007;97:704-13

46. Senior RM, Griffin GL, Huang JS, et al. Chemotactic activity of platelet alpha granule proteins for fibroblasts. J Cell Biol 1983;96:382-85

47. Nedeau AE, Bauer RJ, Gallagher K, et al. A CXCL5- and bFGF-dependent effect of PDGF-B-activated fibroblasts in promoting trafficking and differentiation of bone marrow-derived mesenchymal stem cells. Exp Cell Res 2008;314:2176-86

48. Kajikawa Y, Morihara T, Sakamoto H, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol 2008;215:837-45

49. Nurden AT, Nurden P, Sánchez M, et al. Platelets and wound healing. Front Biosci 2008;13:3532-48

50. Isenberg JS, Frazier WA, Roberts DD. Thrombospondin-1: a physiological regulator of nitric oxide signalling. Cell Mol Life Sci 2008;65:728-42

51. Hsu C-W, Yuan K, Tseng C-C. The negative effect of platelet-rich plasma on the growth of human cells is associated with secreted thrombospondin-1. Oral Sur Oral Med Oral Pathol Oral Radiol Endod 2009;107:185-92

52. Villeneuve J, Block A, Le Bousse-Kerdiles MC, et al. Tissue inhibitors of matrix metalloproteinases in platelets and megakaryocytes: a novel organization for these secreted proteins. Exp Hematol 2009;37:849-56

53. Anitua E, Andia I, Sanchez M, et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human

tendon cells in culture. J Orthop Res 2005;23:281-6

54. Anitua E, Sanchez M, Nurden AT, et al. Reciprocal actions of platelet-secreted TGF-beta1 on the production of VEGF and HGF by human tendon cells. Plast Reconstr Surg 2007;119:950-9
55. Bates DO, Harper SJ. Regulation of vascular permeability by vascular endothelial growth factors. Vascular Pharmacol 2003;39:225-37
56. Anitua E, Sanchez M, Nurden AT, et al. Autologous fibrin matrices: a potential source of biological mediators that modulate tendon cell activities. J Biomed Mat Res A 2006;77:285-93
57. Lyras D, Kazakos K, Verettas D, et al. Immunohistochemical study of angiogenesis after local administration of platelet-rich plasma in a patellar tendon defect. Int Orthop 2010;34:143-8
58. Virchenko O, Genegard M, Aspenberg P. Independent and additive stimulation of tendon repair by thrombin and platelets. Acta Orthop 2006;77:960-6
59. Anitua E, Sanchez M, Zalduendo MM, et al. Fibroblastic response to treatment with different preparations rich in growth factors. Cell Prolif 2009;42:162-70
60. Cheng M, Wang H, Yoshida R, Murray MM. Platelets and plasma proteins are both required to stimulate collagen gene expression by anterior cruciate ligament cells in three dimensional culture. Tissue Eng Part A 2010;16:1479-89
61. Bosch G, van Schie HT, de Groot MW, et al. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: a placebo controlled experimental study. J Orthop Res 2010;28:211-17
62. Randelli PS, Arrigoni P, Cabitza P, et al. Autologous platelet rich plasma for arthroscopic cuff repair. A pilot study. Disabil Rehabil 2008;30:1584-9
63. Filardo G, Kon E, Della Villa S, et al. Use of platelet rich plasma for the treatment of refractory jumper's knee. Int Orthop 2010;34:909-15
64. Everts PA, Devilee RJ, Brown Mahoney C, et al. Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome. A prospective randomized double-blind study. Eur Surg Res 2008;40:203-10
65. Sánchez M, Anitua E, Azofra J, et al. Comparison of surgically repaired Achilles tendon tears using PRGF. Am J Sports Med 2007;35:245-51
66. Kon E, Filardo G, Delcogliano M, et al. Platelet-rich plasma: a pilot study for treatment of jumper's knee. Injury 2009;40:598-603
67. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006;34:1774-8
68. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of autologous platelet concentrate in lateral epicondylitis in a double blind randomized controlled trial. Am J Sports Med 2010;38:255-62
69. De Vos RJ, Weir A, van Schie HTM, et al. Platelet-rich plasma injection for chronic Achilles tendinopathy. JAMA 2010;303:144-9
70. Smidt N, van der Windt DAWM, Assendelft WJJ, et al. Corticosteroid injections, physiotherapy, or a wait-and-see policy for lateral epicondylitis: a randomised controlled trial. Lancet 2002;359:657-62
71. Fufa D, Shealy B, Jacobson M, et al. Activation of platelet-rich plasma using soluble type-I collagen. J Oral Maxillofac Surg 2008;66:684-90
72. Ji Y, Lu Y, Yang F, et al. Acute and gradual increases in BDNF concentration elicit distinct signalling and functions in neurons. Nat Neurosci 2010;13:302-9
73. Humphrey J, Chan O, Crisp T, et al. The short-term effects of high volume image guided injections in resistant non-insertional Achilles tendinopathy. J Science Med Sport 2010;13:295-8
74. Maffulli N, Khan KM, Puddu GC. Overuse tendon conditions: time to change a confusing terminology. Arthroscopy 1998;14:840-3
75. Longo UG, Ronga N, Maffulli N. Achilles tendinopathy. Sports Med & Arthroscop Rev 2009;17:112-26
76. Egerbacher M, Arnoczky SP, Caballero O, et al. Loss of homeostatic tension induces apoptosis in tendon cells. Clin Orthop Relat Res 2008;466:1562-8
77. Yuan J, Murrell GA, Wei AQ, Wang MX. Apoptosis in rotator cuff tendonopathy. J Orthop Res 2002;20:1372-9
78. Lian O, Scott A, Engebretsen L, et al. Excessive apoptosis in patellar tendinopathy in Athletes. Am J Sports Med 2007;35:605-10
79. Pearce CJ, Ismail M, Calder JD. Is apoptosis a cause for non-insertional Achilles tendinopathy? Am J Sports Med 2009;37:2440-4
80. Millar NL, Wei AQ, Molloy TJ, et al. Heat shock protein and apoptosis in supraspinatus tendinopathy. Clin Orthop Relat Res 2008;466:1569-76
81. Scott A, Khan KM, Duronio V. IGF-I activates PKB and prevents anoxic apoptosis in Achilles tendon cells. J Orthop Res 2005;23:1219-25
82. Gagarina V, Carlberg AL, Pereira-Mouries L, Hall DJ. Cartilage oligomeric matrix protein protects cells against death by elevating members of the IAP family of survival proteins. J Biol Chem 2008;283:648-59
83. Longo UG, Franceschi F, Ruzzini L, et al. Light microscopic histology of supraspinatus tendon ruptures. Knee Surg Sports Traumatol Arthroscop 2007;15:1390-4
84. Maffulli N, Longo UG, Franceschi F, et al. Movin and Bonar scores assess the same characteristics of tendon histology. Clin Orthop Relat Res 2008;466:1605-11
85. Scott A, Lian O, Bahr R, et al. VEGF expression in patellar tendinopathy: a preliminary study. Clin Orthop Relat Res 2008;466:1598-604
86. Danielson P, Andersson G, Alfredson H, Forsgren S. Marked sympathetic component in the perivascular innervation of the dorsal paratendinous tissue of the patellar tendon in arthroscopically treated tendinosis patients. Knee Surg Sports Traumatol Arthrosc 2008;16:621-6

87. Divani K, Chan O, Padhiar N, et al. Site of maximum neovascularisation correlates with the site of pain in recalcitrant mid-tendon Achilles tendinopathy. Man Ther 2010: published online 28 April 2010, doi:10.1016/j.math.2010.03.011

88. Brett M, Andres MD, George AC, Murrel MD. Treatment of tendinopathy. Clin Orthop Relat Res 2008;466:1539-54

89. Murrel GAC. Using nitric oxide to treat tendinopathy. Br J Sports Med 2007;41:227-31

90. Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and aetiology with special focus on inflammation. Scand J Med Sci Sports 2008;18:3-15

91. Fedorczyk JM, Barr AE, Rani S, et al. Exposure-dependent increases in IL-1beta, substance P, CTGF, and tendinosis in flexor digitorum tendons with upper extremity repetitive strain injury. J Orthop Res 2010;28:298-307

92. Scott A, Lian O, Bahr R, et al. Increased mast cell numbers in human patellar tendinosis: correlation with symptom duration and vascular hyperplasia. Br J Sports Med 2008;42:753-7

93. Zhang J, Wang JH. Production of PGE2 increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cell into non-tenocytes. J Orthop Res 2010;28:198-203

94. Thampatty BP, Li H, Im HJ, Wang JH. EP4 receptors regulates collagen type-I, MMP-1, and MMP-3 gene expression in human tendon fibroblasts in response to IL-1 beta treatment. Gene 2007;386:154-61

95. Fredberg U, Ostgaard R. Effect of ultrasound-guided, peritendinous injections of adalimumab and anakinra in chronic Achilles tendinopathy: a pilot study. Scand J Med Sci Sports 2009;19:338-44

96. Danielson P. Reviving the "biochemical" hypothesis for tendinopathy: new findings suggest the involvement of locally produced signal substances. Br J Sports Med 2009;43:265-8

97. Bjur D, Danielson P, Alfredson H, Forsgren S. Presence of non-neuronal cholinergic system and occurrence of up- and down-regulation in expression of M2 muscarinic acetylcholine receptors: new aspects and importance regarding Achilles tendon tendinosis. Cell Tissue Res 2008;331:385-400

98. Danielson P, Alfredson H, Forsgren S. Immunohistochemical and histochemical findings favouring the occurrence of autocrine/paracrine as well as nerve related cholinergic effects in chronic painful patellar tendon tendinosis. Microsc Res Tech 2006;69:808-19

99. Bagge J, Lorentzon R, Alfredson H, Forsgren S. Unexpected presence of the neurotrophins NGF and BDNF and the neurotrophin receptor p75 in the tendon cells of the human Achilles tendon. Histol Histopathol 2009;24:839-48

100. Scott A, Bahr R. Neuropeptides in tendinopathy. Front Biosci 2009;14:2203-21

101. Gazerani P, Wang K, Cairns BE, et al. Effect of subcutaneous administration of glutamate on pain, sensitization and vasomotor responses in healthy men and women. Pain 2006;124:338-48

102. Alfredson HS, Forsgren S, Thorsten K, Lorentzon R. In vivo microdialysis and immunohistochemical analyses of tendon tissue demonstrated high amounts of free glutamate and glutamate NMeDAR1 receptors, but not sings of inflammation in Jumper's knee. J Orthop Res 2001;19:881-6

103. Gibson W, Arendt-Nielsen L, Sessle BJ, Graven-Nielsen T. Glutamate and capsaicin-induced pain, hyperalgesis and modulatory interactions in human tendon tissue. Exp Brain Res 2009;194:173-82

104. Schizas N, Lian O, Frihagen F, et al. Coexistence of up-regulated NMDA receptor 1 and glutamate on nerves, vessels and transformed tenocytes in tendinopathy. Scand J Med Sci Sports 2010;20:208-15

Affiliation

Isabel Andia[†1] PhD, Mikel Sanchez[2] & Nicola Maffulli[3]
[†]Author for correspondence
[1]Osakidetza,
Basque Health Service,
Research, B Arteaga 107,
Zamudio 48170, Spain
E-mail: iandia1@hotmail.com
[2]USP Hospitales,
Unidad de Cirugia Artroscópica,
c/La esperanza 3,
Vitoria 01002, Spain
[3]Queen Mary University of London,
Barts and The London School of Medicine and Dentistry,
Centre for Sports and Exercise Medicine,
London, UK