Role of platelet-rich plasma in soft and hard connective tissue healing: an evidence-based review from basic to clinical application

A Moshiri1*, A Oryan2

Abstract

Introduction
Platelet-rich plasma is one of the new therapeutic approaches in the field of soft and hard connective tissue healing and repair. Compared with physiologic plasma, platelet-rich plasma has a higher concentration of platelets with or without a higher concentration of leukocytes. The platelet granules contain many critical growth factors necessary for wound healing. After activation in the injured area, the platelets and leukocytes present in platelet-rich plasma release their growth factors, cytokines and proinflammatory mediators. It is believed that by these mechanisms the platelet-rich plasma initiates inflammation, regulates cell migration, proliferation and matrix deposition and reduces the risk of infection in the injured site. Therefore, it appears that platelet-rich plasma may increase the quality of tissue healing and repair. Several in vitro and clinical trial studies have tested platelet-rich plasma; however, there are significant controversies between their results. This review, introduces platelet-rich plasma, its methods of preparation and benefits. We have also reviewed those researches that tested platelet-rich plasma from basic to the clinic and showed why these controversies exist. This review suggests that the positive effects of platelet-rich plasma were less significant in clinical trials compared with the in vitro and in vivo experimental studies; therefore, it should be highlighted that platelet-rich plasma should not be considered as the first line of treatment in clinics because there is still a lack of evidence demonstrating that the administration of platelet-rich plasma is an effective method to restore the quality and integrity of the injured tissues.

Conclusion
Platelet-rich plasma has some beneficial effects, especially at preclinical level. Most in vitro and in vivo animal studies used allogous forms of platelet-rich plasma, and the results confirmed the efficacy of platelet-rich plasma in soft and hard connective tissue healing in different animal species. In general, platelet-rich plasma is an effective conservative treatment without the complications and cost of surgery, and with moderate success of resolving various musculoskeletal conditions; however, it should not be placed in the first line of treatment modality because it is almost effective in the short term. Future clinical trial studies should be designed to test the real efficacy of platelet-rich plasma in a more standard manner.

Introduction
Treatment of musculoskeletal injuries is often hard to manage, because these soft and hard connective tissues have low vascularity, healing rate and capability and tolerate stressful forces during the healing process, which increase complications1-4. Several reconstructive techniques and tissue engineering approaches have been introduced with the aim to restore the initial function at the injured area25; however, due to the low healing capacity and several well-defined complications such as adhesions in the healing tendons, non-union and osteomyelitis in the healing bones, there is a need for accelerating the healing response in these tissues with the hope to improve the final outcome of the patients26-13. Several healing promotive agents such as glycosaminoglycans and growth factors have been tested in animal studies with this purpose, but due to insufficient related clinical trials their clinical efficacy has not been well approved6–10,14-19.

Platelet-rich plasma (PRP) is one of these healing promotive agents that was introduced several years ago but became popular recently20,21. The efficacy of certain growth factors in healing various injuries and the concentrations of these growth factors found within PRP are the theoretical basis for the use of PRP in tissue repair22. The platelets collected in PRP are activated by addition of thrombin or calcium chloride, which induce the release of these growth factors from α-granules. These growth factors and cytokines include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin-8 (IL-8), keratinocyte growth factor (KGF), and connective tissue growth factor (CTGF)23,21.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

PRP has been tested in vitro and in vivo level in many studies. Most of the in vivo animal studies reported the beneficial effects of PRP in the healing of various soft and hard connective tissues. In humans, PRP has been investigated and used as a clinical tool for several types of medical treatments, including nerve injuries, tendinitis, osteoarthritis (OA), cardiac muscle injury, bone repair and regeneration, plastic surgery, and oral surgery.

It is believed that PRP is effective both in humans and animals; however, several controversies exist between the basic to clinical researches in this regard. In this review, we introduce PRP, explain the methods of its preparation, discuss its mechanisms of action in the healing process and give some definition about its benefits in the field of tissue regenerative medicine. Moreover, we have reviewed several studies from basic to clinical and compared their methodologies and conclusions to find out why these controversies exist in their results.

What is PRP and how is it prepared?
PRP is plasma that contains a higher concentration of platelets than the physiologic plasma. It is obtained from a sample of the patient’s venous blood drawn at the time of treatment. The blood is drawn with the addition of an anticoagulant, such as citrate dextrose A to prevent platelet activation before use. This sample is then placed in a specialised ‘Tabletop’ device that allows for automated separation of the PRP from the platelet-poor plasma (PPP) and the red blood cells (RBCs). On centrifugation of anti-coagulated blood, three layers form, which include: red blood cells (bottom), white blood cells (WBCs)/platelets (buffy coat) (middle) and plasma (top) (Figure 1). The PRP contains a thin layer of concentrated platelets and a “buffy coat” layer with an elevated level of leukocytes (Figure 1). Although it is possible to produce PRP from units of whole blood, most surgical procedures require smaller amounts of PRP. Therefore, several manufacturers have developed technology using small desktop centrifuges (Figure 1) and smaller volumes of blood (45–60 ml) to produce between 5 and 10 ml of PRP. The commercially available PRP kit systems are produced for orthopaedic surgeons. GPS II (Biomet, Warsaw, IN), Symphony II (DePuy), Cascade (Musculoskeletal Transplant Foundation, Edison, NJ), and Magellan (Medtronic, Minneapolis, MN) are examples of these products. These systems consist of a reusable desktop centrifuge and single-use blood collection and tube kits. Most systems rely on an initial low-speed centrifugation to remove red blood cells followed by a high-speed centrifugation to concentrate the platelets. The systems result in a two- to eight-fold increase in platelet concentration compared with the physiologic levels. Although they appear to use similar technologies, these systems are different with respect to the anticoagulant used, final platelet concentration, platelet activation method, method of delivery and level of growth factors released; therefore, further study is clearly warranted to determine the optimal preparation method for orthopaedic applications of PRP. The most common method of platelet activation is the exposure of the PRP to bovine or autologous thrombin, although some systems do not activate platelets before delivery (Figure 1). It is necessary that platelets be activated at the level of tissue injury for the PRP graft to be effective in the healing process. During platelet activation, the platelets release their contents and begin the cascade of events that leads to the restoration and growth of normal tissue.

Normal platelet concentration in the blood is 200,000–300,000 platelets/μl. PRP has been found to contain from four to ten times higher concentration of platelets than that found in whole blood. It is accepted that a PRP graft with platelet count of five- to six-fold greater than the baseline value appears to be adequate to achieve significant outcomes. Many manufacturers promote a high platelet concentration as a reflection of the quality of their device. It must be kept in mind that some studies have indicated that PRP grafts with platelet concentrations greater than eight-fold might have proinflammatory effects leading to inhibition and possible negative outcomes. However, in the literature, a wide range of PRP concentrations have been reported, and the clinical efficacy of PRP has been demonstrated, even with low concentration of PRP.

Once the PRP has been prepared, it is stored in the patient’s operating room at room temperature on a shaker until use. In the surgical field, the PRP is placed in a sterile specimen cup when appropriate during surgery. It can be placed directly into the damaged tissue to initiate and accelerate repair and regeneration. This PRP graft is then activated at the time of injection with addition of baxtroxin, calcium and thrombin (fibrinogen-cleaving agents) or when coming in contact with collagen. The PRP platelets release their platelet growth factors, and other active substances, upon activation (Table 1). Subsequently, fibrin is formed; platelets are activated, leading to platelet degranulation and growth factor release. Successful placement of the graft into the exact location of damage is necessary for optimal results. This application can be accomplished in the office setting by employing needle-guided radiological visualisation of accurate placement (ultrasound (US), fluoroscopy, computed tomography and magnetic resonance imaging (MRI)), and in the operative setting via open or arthroscopic techniques.
Competing interests: none declared.

Review

All authors contributed to the concept, design, preparation of the manuscript, as well as read and approved the final manuscript.

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)

tissue increases. The final phase of healing can last for months or even for years\textsuperscript{53}.

**Why is PRP beneficial in wound healing?**

In the recent years, several studies have revealed a complex regulation of growth factors for the normal tissue structure and reaction to tissue damage and an important role and effectiveness in using growth factors for healing the damaged tissue\textsuperscript{1,3,4,67–69}. Therefore, use of growth factors is thought to be beneficial in clinical practice, as it promotes rapid healing with a high-quality tissue and allows an early and safe return to unrestricted activity. PRP is a simple, cost-effective and minimally invasive way to obtain a natural concentration of autologous growth factors and is currently widely experimented in different fields of medicine for its ability to aid regeneration of tissues with a low healing potential\textsuperscript{67}.

These growth factors are mainly placed in platelets than leukocytes and therefore the role of platelets in the PRP should be highlighted. These growth factors are reserved in α-granules\textsuperscript{67}. α-Granules are also sources of cytokines, chemokines and many other proteins variously involved in stimulating chemotaxis, cell proliferation and maturation, modulating inflammatory molecules and attracting leukocytes\textsuperscript{47,52,55}. In addition, platelets store antibacterial and fungicidal proteins to prevent infections, proteases such as metalloprotease-4 and coagulation factors. Platelets also contain dense granules, which store and release, upon activation, adenosine diphosphate, adenosine triphosphate, calcium ions, histamine, serotonin and dopamine. Moreover, platelets contain lysosomal granules that can secrete acid hydrolases, cathepsin D and E, elastases and lysozyme and most likely other not yet well-characterised molecules, whose role in the tissue healing process should not be underestimated\textsuperscript{2}.

**Mechanism of PRP in wound healing**

The basic mechanism of action of PRP in wound healing is simple. After injection of PRP in an injured area, it induces a local inflammation. The proinflammatory mediators together with the growth factors release from the granules of the cellular structures (platelets, neutrophils, monocytes and lymphocytes) in PRP as described. These growth factors include PDGF, VEGF, FGF, EGF, KGF, IGF-1, IGF-2, TGF-β, CTGF, EGF.

---

Table 1. Growth factors present in platelets.

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transforming growth factor-β (TGF-β)\textsuperscript{91,92}</td>
<td>Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/monocytes and neutrophils</td>
<td>Stimulates undifferentiated mesenchymal cell proliferation, regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation; regulates balance between fibrosis and myocyte regeneration; regulates mitogenic effects of other growth factor</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (bFGF)\textsuperscript{93,94}</td>
<td>Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts</td>
<td>Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal stem cells, chondrocytes, and osteoblasts; stimulates angiogenesis; stimulates proliferation of myoblasts</td>
</tr>
<tr>
<td>Platelet-derived growth factor (PDGF)\textsuperscript{92,95}</td>
<td>Platelets, osteoblasts, endothelial cells, monocytes, smooth muscle cells</td>
<td>Mitogenic for mesenchymal stem cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glia/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis, stimulates angiogenesis</td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)\textsuperscript{96,97}</td>
<td>Platelets, macrophages, monocytes</td>
<td>Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal/chondrocytes/osteoblasts mitogenesis; promotes growth/differentiation of chondrocytes and osteoblasts</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)\textsuperscript{98,99}</td>
<td>Platelets, endothelial cells</td>
<td>Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells</td>
</tr>
<tr>
<td>Connective tissue growth factor (CTGF)\textsuperscript{100}</td>
<td>Platelets through extracellular matrix from extracellular environment in bone marrow</td>
<td>Promotes angiogenesis, cartilage regeneration, fibrosis, and platelet adhesion</td>
</tr>
</tbody>
</table>
and IL-8. The role of each growth factor has been presented in Table 1. The localised inflammation and the released growth factors trigger the wound healing cascade, resulting in the cellular migration and proliferation, glycosaminoglycan and collagen deposition, collagen maturation and remodelling of the healing tissue at different stages of wound healing.52,72.

The neutrophils and monocytes contain granules filled with myeloperoxidase, a substance that contributes to the antimicrobial activity of platelet-rich gel at the site of application.52

How do the PDGFs regulate wound healing?

As shown in Table 1, tissue repair initially begins with the activation of coagulation cascade, platelet clot formation, platelet aggregation and degranulation. During this degranulation period, the platelets release a pool of biologically active proteins and other substances into the extracellular environment. The biologically active proteins might bind to specific platelet growth factor receptors present in surgical tissues. Released growth factors interact and bind with the platelet tyrosine kinase receptor (TKR), which is present in the cell membranes of tissue cells (ligand–receptor interaction).52 Therefore, the actual binding site is on the external surface of the cell membrane, and thus is not directly on the cell nucleus. The TKR is a membrane spanning protein that extends into the cytoplasm of cells. After the platelet growth factor interacts with the external part of the TKR, activation of inactive messenger proteins occurs in the cytoplasm. The messenger proteins then become activated and bind to the TKR cytoplasmic tail. Activated proteins are generated via an active signalling cascade in the cell nucleus where the genes responsible for control of cell division are triggered. Thus, transcription of messenger RNA is induced, producing a biological response that starts cascades, which in turn provokes tissue repair and tissue regeneration.52 Although secretion of growth factors occurs mainly in the first hour, it has been observed that platelets remain viable for 7 days and continue to release growth factors, suggesting that one single injection into the damaged tissue might be a sufficient treatment in most clinical applications.50,70–72

In vitro studies

Several in vitro studies have shown the role of PRP on cell migration, proliferation and viability; however, some of them have reported adverse effects of this reagent in different cells. Generally, at in vitro level, the reports showing the beneficial effect of PRP on cell migration, proliferation and viability are predominant. Here, the effect of PRP on the stem cells, fibroblasts, tenocytes, chondrocytes and synoviocytes has been discussed.

Effects of PRP on stem cells

One of the beneficial effects of PRP is its ability to induce cellular migration, differentiation and maturation in stem cells. Perhaps this ability could have a beneficial role in the treatment of injured tissues. Zhang et al.73 determined the effects of PRP, in the form of PRP-clot releasate (PRCR), on tendon stem cells (TSCs). Their in vitro results indicated that the TSCs without PRCR treatment were small and exhibited irregular shape, whereas with increasing PRCR dosage, TSCs became large, well spread and highly elongated with down-regulation of nucleostemin expression. The PRCR treatment also markedly enhanced the TSC proliferation, tenocyte-related gene and protein expression and total collagen production, all of which indicated that PRCR treatment induced differentiation of TSCs into activated tenocytes. The findings of this study suggested that PRP treatment of injured tendons is ‘safe’, as it promotes TSC differentiation into tenocytes rather than non-tenocytes, which would compromise the structure and function of healing tendons by formation of non-tendinous tissues.74 Moreover, they suggested that PRP treatment could enhance tendon healing because the tenocytes induced to differentiate by PRP are activated to proliferate quickly and produce abundant collagen in the healing of injured tendons that have lost cells and matrix. The major merit of their study was that they used several molecular to morphological observations to define the efficacy of the PRP in vitro. They also counted the platelet number in their PRP samples and it was 1.44 × 10⁸ to 0.84 × 10⁹/µl. This concentration is a high dose of platelets. It appears that the higher concentration of platelets may have more beneficial effects than the lower one. However, one of the limitations of the study of Zhang et al.73 was that their study was an in vitro investigation and despite applying several methodologies their results cannot be suggested for clinical practice or an in vivo situation. Because the in vitro studies fail to design the inflammatory condition ex vivo and due to the inflammatory reaction of PRP in vivo, it is better to test similar studies with the same methodologies used at in vivo level.

As stated earlier, PRP has two forms, solution (inactivated) and gel (activated) forms. It appears that the gel form of PRP is more effective in releasing growth factors in vitro. In another study, Wang et al.75 compared release of growth factors between platelet-rich fibrin (PRF) and PRP as well as their effects on proliferation and differentiation of adipose tissue-derived stem cells (ADSCs) in vitro. For this purpose, they harvested blood from the central artery of rabbits, acquiring PRF was done through one time centrifuge and PRP through two times centrifuge. Five millilitres of fresh α-minimum essential medium was added to PRF and PRP and incubated at 37°C. The time points to collect exudates were on day 1, 7, 14, 21, 28 and the mass concentrations of TGF-β1 and

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)

PDGF-AB were quantified in PRF and PRP. The exudates of PRF and PRP were then used to culture ADSCs and evaluate the effects of PRF and PRP on proliferation and differentiation of ADSCs. Their results indicated that PRF released growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of ADSCs, as compared with PRP.

Effects of PRP on fibroblasts and tenocytes

Tendon and ligaments have a low healing capability, and for this reason the tenocytes are one of the main cells which are tested in vitro. The efficacy of PRP on tenocytes and fibroblasts has been widely investigated at in vitro level and is mainly related to cell proliferation, migration and adhesion. Anitura et al.66 evaluated the biological effects of PRP on primary human periodontal ligament fibroblasts. Their results indicated that the autologous PRP significantly stimulated cell proliferation, migration and adhesion. A synthesis of many growth factors from cells including VEGF, thrombospondin 1, CTGF, hepatocyte growth factor and pro-collagen type I was also stimulated by autologous PRP. They concluded that the PRP had positive effects on periodontal ligament fibroblasts, which could be positive for periodontal regeneration. In another study, Tohidnezhad et al.77 investigated the effect of platelet-released growth factors on tenocytes that were isolated from the Achilles tendon of postnatal rats, in vitro. They showed that these growth factors promoted the tenocyte growth and activated the Nrf2-ARE pathway. Visser et al.78 compared the concentration of a representative TGF-β1 eluted from a PRF matrix, a PRF membrane and a whole blood clot over time, and also compared the mitogenic effect of the eluents from each construct, in vitro. Their results indicated that both PRF matrix and PRF membrane comprised of a dense fibrin scaffold that contained increased concentrations of TGF-β1 and they were capable of increasing the tendon cell proliferation over time when compared with a whole blood clot.

PRP has been shown to have some more advantageous roles. Muto et al.79 investigated the role of PRP on the protection of the human rotator cuff-derived cells after administration of triamcinolone acetonide (TA) and indicated that the deleterious effect of TA was prevented by PRP, which can be used as a protective agent for patients receiving local TA injections.

Effects of PRP on chondrocytes and synoviocytes

Although it appears that PRP may have a role in reducing the signs of osteoarthrosis, some in vitro studies suggested that this therapeutic agent may be harmful for the cartilage. Browning et al.80 studied the effect of PRP on chondrocytes in cell and tissue culture and showed that PRP contains a mixture of anabolic and catabolic mediators. The PRP cultured in medium contained multiple catabolic mediators in substantial concentrations, including matrix metalloproteinase (MMP)-9 and MMP-1 as well as proinflammatory mediators IL-1β; IL-6; interferon-γ; IP-10; monocyte chemotactic protein-1; macrophage inflammatory protein-1β; regulated on activation, normal T cell expressed and secreted and tumour necrosis factor-α. PPP contained significantly lower concentrations of these compounds. PRP was used to treat human fibroblast-like synoviocytes. Compared with untreated fibroblast-like synoviocytes, the synoviocytes that were treated with PRP exhibited significantly greater levels of MMP-1 and MMP-3. PPP had little effect on mediators secreted by the synoviocytes. They concluded that the synoviocytes treated with PRP responded with substantial MMP secretion, which may increase cartilage catabolism. Synoviocytes responded to PDGF with a substantial proinflammatory response.

Animal studies

An important stage of basic to clinical translation is the animal studies. In fact, in an in vivo situation several uncontrolled events can influence the efficacy of any treatment modality. One of these events is the host’s immune response to graft, which is initiated after treatment of the injured area with any exogenous agent. Therefore, by designing a proper methodology, a better judgment can be made about the real efficacy of the treatment modality than in vitro situations. Several in vivo animal studies have tested the efficacy of PRP, in its solution or gel forms, and unlike clinical trial studies, tested PRP in humans, the methodologies are more homogenous and standard, the treatment modality is tested in different species of animals and therefore, the results of the in vivo animal studies are more reliable to understand and to conclude. Despite these explanations, it should be remembered that the animal studies are an approximation, and several differences between the human and animals’ anatomy and physiology should be considered when the results of animal studies are going to be translated for clinical practice.

Soft connective tissue (tendon and ligament)

As has been discussed earlier, tendon and ligaments have a low healing capability and their treatment generally is hard to manage. Therefore, several studies tested the efficacy of PRP in experimentally induced tendon or ligament injuries to show whether PRP is effective in restoring the structural and functional characteristics of the injured tissue. Most of the in vivo animal studies, regardless of the methods of PRP preparation suggested PRP as one of the effective treatment options. Here, we discuss about these studies based on the animal species they have used. The
Table 2. Effects of PRP or PRP gel in different animal experimental studies.

<table>
<thead>
<tr>
<th>No</th>
<th>Reference</th>
<th>Model of injury</th>
<th>Animal model</th>
<th>Effective/ non-effective/ harmfull</th>
<th>Types of the effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspenberg and Virchenko22</td>
<td>Achilles tendon defect model</td>
<td>Rat</td>
<td>Effective</td>
<td>Improved the structural and biomechanical properties of the tendon callus</td>
</tr>
<tr>
<td>2</td>
<td>Spang et al.24</td>
<td>Patellar tendon transection model</td>
<td>Rat</td>
<td>Non-effective</td>
<td>No effects on biomechanical properties</td>
</tr>
<tr>
<td>3</td>
<td>Wnuk et al.25</td>
<td>Achilles tendon transection model</td>
<td>Rat</td>
<td>Effective</td>
<td>Increased the cellular and vascular density with an improved fibre alignment</td>
</tr>
<tr>
<td>4</td>
<td>Beck et al.26</td>
<td>Supraspinatus tendon tear</td>
<td>Rat</td>
<td>Non-effective</td>
<td>Increased the alignment of the collagen fibres but did not improve the tensile load</td>
</tr>
<tr>
<td>5</td>
<td>Kaux et al.27</td>
<td>Achilles tendon transection model</td>
<td>Rat</td>
<td>Effective</td>
<td>Increased tenomodulin and collagen type I</td>
</tr>
<tr>
<td>6</td>
<td>Barbosa et al.28</td>
<td>Tenotomy</td>
<td>Rat</td>
<td>Effective</td>
<td>Increased the deposition of collagen type I</td>
</tr>
<tr>
<td>7</td>
<td>Sato et al.29</td>
<td>Intra-synovial flexor tendon transection model</td>
<td>Rabbit</td>
<td>Non-effective</td>
<td>Had no effect when used as an alone treatment</td>
</tr>
<tr>
<td>8</td>
<td>Harris et al.30</td>
<td>Normal soft tissue (to induce inflammation)</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased the acute inflammatory response</td>
</tr>
<tr>
<td>9</td>
<td>Dragoo et al.31</td>
<td>Normal tendon (to induce inflammation)</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased the acute inflammatory response</td>
</tr>
<tr>
<td>10</td>
<td>Lyras et al.32</td>
<td>Achilles tendon transection model</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased neovascularisation and fibre alignment</td>
</tr>
<tr>
<td>11</td>
<td>Lyras et al.33</td>
<td>Patellar tendon injury</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased the biomechanics of the repaired tendons</td>
</tr>
<tr>
<td>12</td>
<td>Hernández-Martínez et al.34</td>
<td>Achilles tendon tear</td>
<td>Dog</td>
<td>Effective</td>
<td>Increased angiogenesis and fibroblasts proliferation</td>
</tr>
<tr>
<td>13</td>
<td>Xie et al.35</td>
<td>ACL reconstruction</td>
<td>Dog</td>
<td>Effective</td>
<td>Increased the necessary gene expression of the extra cellular matrix and regeneration</td>
</tr>
<tr>
<td>14</td>
<td>Sarrafian et al.36</td>
<td>Acute Achilles tendon rupture</td>
<td>Sheep</td>
<td>Effective</td>
<td>Increased the quality of new tendon formation in a gap</td>
</tr>
<tr>
<td>15</td>
<td>Bosch et al.37</td>
<td>Tendon injury</td>
<td>Horse</td>
<td>Effective</td>
<td>Increased neovascularisation</td>
</tr>
<tr>
<td>16</td>
<td>Kim et al.38</td>
<td>Achilles tendon-calcaneus bone junction</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased the biomechanical properties and alignment of the healing tendons</td>
</tr>
<tr>
<td>17</td>
<td>Hapa et al.39</td>
<td>Tendon-to-bone healing in a rotator cuff repair model</td>
<td>Rat</td>
<td>Effective</td>
<td>Effective in tendon healing and bone remodelling</td>
</tr>
<tr>
<td>18</td>
<td>Gumieiro et al.40</td>
<td>Circular defects created in irradiated tibiae</td>
<td>Rat</td>
<td>Effective</td>
<td>Cell regeneration and bone formation</td>
</tr>
<tr>
<td>19</td>
<td>Nather et al.41</td>
<td>Tibial defect reconstruction</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Increased bone union and bone resorption</td>
</tr>
<tr>
<td>20</td>
<td>Li et al.42</td>
<td>Tibial osteomyelitis</td>
<td>Rabbit</td>
<td>Effective</td>
<td>Anti-microbial</td>
</tr>
</tbody>
</table>
summary of their model of injury, effectiveness or non-effectiveness of PRP and the factor that was influenced by PRP has been provided in Table 2.

Rat

From the authors’ experience, the rat is one the most tested animal species and used as an animal model to define the efficacy of different treatment modalities. However, the rat is not a proper animal model for tendon and ligament injuries because their tendon or ligament structures are too small and therefore designing a well-matched model of tendon or ligament injury is hard. Thus, the generalisation of the results obtained from this animal species to the clinical situation is slightly difficult. Despite these explanations, most of the studies suggested PRP as an effective treatment modality in rats.

Aspenberg and Virchenko22 studied whether a platelet concentrate injection would improve Achilles tendon repair in an established rat model. The Achilles tendon was transected and a 3 mm segment was removed. After 6 h, a platelet concentrate was injected percutaneously into the haematoma. This increased tendon callus strength and stiffness by about 30% after 1 week, which persisted for as long as 3 weeks after the injection. At this time, the mechanical testing indicated an improvement in material characteristics, i.e. greater maturation of the tendon callus. This was confirmed by blinded histological scoring. They used allogous PRP because they prepared a PRP from three donor rats and used it for 10 rats. This model of PRP preparation is not popular in clinical practice but their results showed that the allogous-based PRP is effective in the Achilles tendon defect model in rats; however, they failed to design a control group to be treated with autogenous PRP to show whether the allogous based PRP was effective as the autogenous PRP. Also, they only tested their samples with biomechanical and qualitative histological methodologies and failed to analyse the healing tissues in a more detailed manner. Therefore, it can be suggested that this study failed to prove the real efficacy of PRP on tendon healing; however, their results were encouraging.22 Actually this is one of the first researches that motivated researchers to use PRP as one of the treatment modalities in their researches.

In another study, Spang et al.24 evaluated the effects of a single centrifuge platelet concentrate on tendon healing in rats. The rats had a surgical transection of the patellar tendon that was subsequently stabilised with a cerclage suture. Prior to skin closure, the tendon was saturated with either a concentrated platelet solution or saline. At 14 days, all animals were euthanised, and the extensor mechanism was isolated for biomechanical testing. Comparisons between the control group and the concentrated platelet group revealed no differences. Although the results of Spang et al.’s24 study was negative, they showed that a single dose of PRP preparation, because of low concentration of platelets, is not effective, and this indicates that it may be more valuable to prepare a PRP by double-step method (two steps of centrifugation). In another study, Wnuk et al.25 investigated the potential impact of PRP on tendon healing in 88 rats. The animals were randomly divided into two groups. The animals from the examined group (n = 44) with an operated calcaneal tendon were given subcutaneously allogeneic PRP. The animals from the control group (n = 44) were given 0.9% NaCl solution within the area of calcaneal tendon damage. After 7, 14, 21 and 42 days, the tendons were tested mechanically and were subjected to histological evaluation. They indicated that the tendons of the examined groups were characterised by higher cellular and vascular density and a more orderly arrangement of collagen fibres compared with the control groups. They concluded that PRP increased the mechanical strength and histological maturity of the regenerating calcaneal tendons after 14, 21 and 42 days from injury.

In another study, Kaux et al.27 determined that an injection of PRP could improve the healing potential of the sectioned Achilles tendons of rats. After surgery, the rats received an injection of PRP (n = 60) or a physiological solution (n = 60) in situ. After 5, 15 and 30 days, the rats of both groups were euthanised. The tendons of the PRP group were more resistant to rupture at 15 and 30 days. The mechanical stress was significantly higher in the tendons of the PRP group at day 30. The histological analysis showed a precocious deposition of fibrillar collagen at day 5 confirmed by a biochemical measurement. The expression of tenomodulin was significantly higher at day 5. The messenger RNA levels of type III collagen, matrix metalloproteinases 2, 3 and 9 were similar in the two groups at all time-points, whereas type I collagen was significantly greater at day 30 in the PRP group. They concluded that an injection of PRP in the sectioned Achilles tendon of rat influences the early phase of tendon healing and results in an ultimately stronger mechanical resistance. These results had a major clinical relevance because most of the clinical researches suggested that PRP is effective in the early period of healing process.26-66

Barbosa et al.28 investigated the effects of low-level laser therapy treatment alone or associated with PRP, in rats. They euthanised their animals on the 13th day post-tenotomy and their tendons were surgically removed for a quantitative analysis using polarised microscopy. Their results showed that the deposition of collagen type I was higher, suggesting a faster regeneration of the tendon, when treatment with PRP and low-level laser therapy was combined.
Other researchers tested different tendon injury models based on the anatomic position. Beck et al.\textsuperscript{26} tested the effects of PRP on rotator cuff repair in rats. They used an allogenic form of PRP. A tendon-from-cortical supraspinatus tear was created surgically and an immediate transosseous repair performed. The control group underwent repair only. The PRP group underwent a repair with PRP augmentation. The rats of each group were sacrificed at 7, 14 and 21 days. Their results indicated that at 7- and 21-day augmentation with PRP showed statistically significant effects on the biomechanical properties of the repaired rat supraspinatus tear, but the failure load was not increased at the 7, 14 or 21-day period. The control group had significantly higher stiffness at 21 days. The control group had a higher failure strain at 7 days, whereas the PRP group had a higher failure strain at 21 days. On histological examination, the PRP group showed increased fibroblastic response and vascular proliferation at each time point. At 21 days, the collagen fibres in the PRP group were orientated in a more linear fashion toward the tendon footprint. They concluded that PRP altered the tissue properties of the supraspinatus tendon without affecting the construct’s failure load.

**Rabbit**

Rabbit is a better experimental model of tendon injuries because their handling is much easier and their tendons and ligaments are larger than rats, thus a better designing for the injury model can be made. The methodologies and results between the rat- and rabbit-based studies are the same and those investigated the efficacy of PRP, suggested it as an option, too. Sato et al.\textsuperscript{29} investigated the effects of autologous PRP in normal soft tissues in rabbits. They injected the PRP in the quadriceps muscle, Achilles tendon, medial collateral ligament, subcutaneous tissue, tibial periostium and ankle joint of the animals. Saline solution was injected on the contralateral side as a control. Their results indicated that PRP could initiate an inflammatory response in the absence of an inciting injury in normal soft tissues in rabbits. In a similar study, Dragoo et al.\textsuperscript{31} evaluated the inflammatory effect of two different commercially available PRP systems, Biomet GPS III leukocyte-rich PRP versus MTF Cascade leukocyte-poor PRP, after intra-tendinous injection in an animal model. Their results indicated that compared with leukocyte-poor Cascade PRP, leukocyte-rich GPS III PRP caused a significantly greater acute inflammatory response at 5 days after injection.

One of the mechanisms of PRP in wound healing is its efficacy in inducing inflammation. Harris et al.\textsuperscript{30} investigated the effects of autologous PRP in normal soft tissues in rabbits. They injected the PRP in the quadriceps muscle, Achilles tendon, medial collateral ligament, subcutaneous tissue, tibial periostium and ankle joint of the animals. Saline solution was injected on the contralateral side as a control. Their results indicated that PRP could initiate an inflammatory response in the absence of an inciting injury in normal soft tissues in rabbits. In a similar study, Dragoo et al.\textsuperscript{31} evaluated the inflammatory effect of two different commercially available PRP systems, Biomet GPS III leukocyte-rich PRP versus MTF Cascade leukocyte-poor PRP, after intra-tendinous injection in an animal model. Their results indicated that compared with leukocyte-poor Cascade PRP, leukocyte-rich GPS III PRP caused a significantly greater acute inflammatory response at 5 days after injection.

One of the mechanisms of PRP in wound healing is related to angiogenesis. Lyras et al.\textsuperscript{32} studied the effect of PRP gel on the mechanical properties of the rabbit’s patellar tendon after transecting its central portion. Forty rabbits in four groups of 10 rabbits each (two groups of PRP and two groups of control) were used to evaluate the mechanical properties and histology after 14 and 28 days of injury. Their results indicated that the mechanical properties of the regenerated tendon in the PRP group were significantly improved compared with the control group. They concluded that PRP has a strong effect on the early phase of tendon healing.

**Dog**

Canines are much more suitable models of animal studies. Their injuries and anatomy are more similar to humans than rats or rabbits. Their tendons, ligaments and bones are larger and more appropriate models of injury can be designed in this species. Canine studies also suggest PRP as an effective treatment modality in soft tissue repair.

The angiogenic effects of PRP have been shown in canine-based studies too. Hernández-Martínez et al.\textsuperscript{34} reported studies, it appears that PRP is effective in the early stages of wound healing. Lyras et al.\textsuperscript{33} studied the effect of PRP gel on the mechanical properties of the rabbit’s patellar tendon after transecting its central portion. Forty rabbits in four groups of 10 rabbits each (two groups of PRP and two groups of control) were used to evaluate the mechanical properties and histology after 14 and 28 days of injury. Their results indicated that the mechanical properties of the regenerated tendon in the PRP group were significantly improved compared with the control group. They concluded that PRP has a strong effect on the early phase of tendon healing.
compared the functional and histological course of two animal model groups with acute Achilles tendon tears using PRP. An open clinical trial was conducted with dogs. Dogs were divided into two groups: a control group and a problem group. Intentional surgical Achilles tendon tear was performed. The Krackow technique was used to repair the tendon and the problem group received PRP as a clot; the other group did not receive PRP. The dogs were observed at 4 weeks to check functionality using the Farell and Schwarz scale to assess the degree of limping. They were euthanised at week 5, and the tendons were removed for histopathological studies. Functionality results showed grades I and II in the problem group, and grades IV and V in the control group. Histological examination revealed the problem group with moderate vascular proliferation and abundant fibroblastic proliferation. The control group had mild to moderate vascular proliferation and moderate fibroblastic proliferation. They concluded that the PRP improves tendon healing.

In a different model of injury, Xie et al.35 investigated the influence of PRP on the synthesis and degradation of the extracellular matrix during the anterior cruciate ligament (ACL) graft remodelling process in a dog model. They designed four groups of treated (PRP), control (saline), sham (only the knee joints were exposed) and normal control group (no surgery was performed in the knees). The ligament was dissected at 2, 6 and 12 weeks after surgery, and tested for molecular analyses. Their results indicated that during the graft remodelling process, a time-dependent change in gene expression following ACL reconstruction surgery was observed. In addition, PRP altered the expression of some target genes at certain time points, especially during the early stages of graft remodelling, which might explain the enhancing effect of PRP on the ACL graft maturation process.

**Sheep**

Sheep model of soft connective tissue injury has similar advantages to those of the canine model; however, the prevalence of sheep-based animal studies is lower than those of the canine model. In this model, some investigators confirm the efficacy of PRP. Sarrafian et al.36 evaluated a cross-linked acellular porcine dermal patch (APD), as well as PRP fibrin matrix (PRPFM), in repair of acute Achilles tendon rupture in a sheep model. The two surgically transected tendon ends were re-approximated in groups 1 and 2, whereas a gap was left between the tendon ends in group 3. APD was used to reinforce the repair in group 2, and autologous PRPFM was used to fill the gap, which was also reinforced with APD, in group 3. All sheep were euthanised at 24 weeks after surgery and biomechanical and histological testing were performed. Their results indicated a statistically significant difference in elongation between the operated limb and the un-operated contralateral limb in groups 1 and 3, but not in group 2. All operated tendons appeared healed with no apparent fibrosis under light and polarised microscopy. In group 1, all surgical separation sites were identifiable, and healing occurred via increasing tendon thickness. In group 2, healing occurred with new tendon fibres across the separation, without increasing tendon thickness in two out of six animals. Group 3 showed complete bridging of the gap, with no change in tendon thickness in two out of six animals. In groups 2 and 3, peripheral integration of the APD to tendon fibres was observed. They suggested the use of APD, alone or with PRPFM, to augment Achilles tendon repair in a sheep model. However, they failed to report whether the PRP is effective alone and this should be considered as one of the limitations of their study.

**Horse**

Horse is not a well-accepted experimental model for human medicine; however, this animal is very important in the field of veterinary medicine because tendon and ligament injuries are very prevalent in this species and this animal species is presented frequently in veterinary practice. PRP has been shown to increase angiogenesis in horses. Bosch et al.37 studied the effect of PRP on neovascularisation in experimentally induced tendon injury using colour Doppler ultrasonography and immunological staining for Factor VIII in an equine model. PRP induced significantly more neovascularisation than the placebo treatment until at least 23 weeks after treatment, as detected by both Doppler ultrasonography and Factor VIII staining. Neovascularisation might be one of the explanations for the long-lasting effect of a single intra-tendinous treatment with PRP.

**Effects of PRP on bone repair**

Treatment of hard tissue is also challenging. Several factors influence the healing of these tissues and increase the risk of non-union, delayed union, osteomyelitis and other problems. PRP has been suggested to be effective in healing injured hard tissues in different species of animals. Kim et al.38 determined whether exogenously injected bone marrow-derived PRP plus (BMP)-2 could accelerate the healing of bone–tendon junction injuries and increase the junction holding strength during the early regeneration period. A direct injury model of the bone–tendon junction was made using an Achilles tendon–calcaneus bone junction in a rabbit model. In the PRP/BMP-2/fibrin group, 0.05 ml of bone marrow-derived PRP and 100 ng/ml of BMP-2, both incorporated into 0.1 ml of fibrin glue, were injected into the Achilles tendon-calcaneus bone junctions. Histological examinations showed that woven bone developed in tendon–bone junctions at 2 weeks after surgery in the PRP/BMP-2/fibrin group. The mechanical test results showed no significant differences compared to the BMP-2/fibrin group.

**Commercial interests: none declared. Conflict of interests: none declared.**

All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
difference between the PRP/BMP-2/fibrin and control groups at 2 and 4 weeks after surgery, but the mean maximal load in the PRP/BMP-2/fibrin group was significantly higher than the control group at 8 weeks after surgery. Their results demonstrated that the PRP might increase the healing quality of the tissue.

In another study, Hapa et al. investigated the effects of local autologous PRP injection on tendon-to-bone healing in a rotator cuff repair model in rats. Rotator cuff injury was created in 68 left shoulders of rats. PRP was obtained from the blood of an additional 15 rats (an allologous form of PRP). The 68 rats were divided into four groups with 17 rats in each group; PRP group (week 2), control group (week 2), PRP group (week 4), and control group (week 4). PRP or saline was injected into the repair area intra-operatively. The animals were euthanised 2 and 4 weeks after the surgery. Their results indicated that the degree of inflammation and vascularity were less in the study group at both time intervals ($p < 0.05$). Tendon continuity was better in the study group at 2 weeks ($p < 0.05$). Obvious new bone formation was detected in the control group at 4 weeks ($p < 0.05$). Biomechanical examination revealed that PRP-treated specimens were stronger at 2 weeks ($p < 0.05$). They concluded that the local autologous PRP injection might have beneficial effects on initial rotator cuff tendon-to-bone healing and enhance initial tendon-to-bone healing remodeling. Although they stated that their PRP was an autologous form, their methods indicated that it was an allologous form and therefore their results could not be concluded for the autologous PRP. Also, they stated that PRP reduced vascularity and the readers may get confused about this.

From the authors’ experience, angiogenesis and neovascularisation are increased at earlier stages of wound healing but as the healing process continues, vascularity decreases. We have discussed this phenomenon in our previous review.

Some researchers tested the efficacy of PRP in different models of bone defects in animal studies. Gumieiro et al. evaluated the influence of PRP on bone repair of circular defects created in irradiated tibiae of rats by histometric analysis. They irradiated the tibiae of the rats with 30 Gy. After 30 days, monocortical defects were created and PRP was applied in 30 rats. In the control group, the defects were created but not filled. They studied the healing pattern of the newly regenerated tissue in the defect area at 4, 7, 14, 21, 56 and 84 days after injury by only histology. They concluded that addition of PRP had a beneficial effect in the initial cellular regeneration period and enhanced bone formation in later periods when compared with the control. Again, the method of preparation of PRP in this study was the same as those Aspenberg and Virchenko. They used two donor animals to get PRP and used it for 10 recipient animals. Thus, their PRP was not an autogenous based and was allologous. One of the major merits of their study was that they counted the platelets in their PRP solution and reported that it was around 400% of the peripheral blood platelet count or 1,000,000 platelet/µl in a volume of 5 ml. However, the only histological evaluation in their study made the impact of their study low and therefore their results should be cautiously considered as a positive report.

In another study, Nather et al. evaluated the effect of autogenous PRP for fresh-frozen allografts in tibial defect reconstruction in rabbits. Thirty-six rabbits underwent tibial defect reconstruction with autografts ($n = 12$), allografts without PRP ($n = 12$) or allografts with PRP ($n = 12$) and were observed for 12, 16 and 24 weeks. Seven millilitres of whole blood was drawn to prepare 1 millilitre of PRP. The PRP was then mixed with 1.0 ml of human thrombin to form a platelet gel. The PRP gel was then packed into the medullary canal of the allograft and applied on the cortical surface before tibial defect reconstruction. Osteo bridging the gap at host–graft junctions was noted in all specimens in the autograft and allograft-with-PRP groups at week 12 and in the allograft-without-PRP group at week 24. Bone union in allografts without PRP was delayed. All indices for biological incorporation (resorption index, new bone formation index, callus encasement index and viable osteocyte count) were significantly greater in the autograft than allograft-without-PRP groups, except for the resorption index at week 24, whereas the differences were not significant between the autograft and allograft-with-PRP groups. The differences between the two allograft groups were usually not significant, except for the resorption index. They concluded that the PRP-augmented allografts behaved similar to the autografts for tibial defect reconstruction in rabbits. PRP increased bone union and bone resorption. One of the limitations of their study was that they did not biomechanically test the healing tissues and therefore, their positive results should be confirmed by biomechanical based studies. Oryan et al. examined the effect of a combination of hydroxyapatite and human PRP on osteogenesis in vivo, using rabbit model bone healing. A critical size defect of 10 mm long was created in the radial diaphysis of 36 rabbits and either supplied with hydroxyapatite-human PRP or hydroxyapatite was left empty (control group). They tested the healing tissues by various methodologies including radiography, histology and biomechanical testing. Their results suggested that addition of PRP to hydroxyapatite increased bone regeneration and failure strength of the healing tissue compared with the control lesions. Their reagent was xenogeneic-based PRP obtained from humans.
results supported this hypothesis that the major mechanism of PRP in wound healing may correlate to its role in inducing inflammation. Comparison between the animal studies reveals that the allogous form of PRP is more immunogenic than the autogenous form and the xenogenous PRP is much more immuno-potent than those of the auto or allo forms. This mechanism may explain why the results of the animal studies are quite different from the human studies.

One of the well-approved effects of PRP is its anti-microbial activity. Li et al.42 investigated the antibacterial property of L-PRP gel against methicillin-resistant Staphylococcus aureus (MRSA, ATCC 43300) in a rabbit model of tibial osteomyelitis. The antibacterial efficacy was evaluated by radiological, microbiological, and histological examinations. Newly formed bone was also quantified. The best therapeutic efficacy, including infection elimination and bone defect repair, was observed in the L-PRP gel + antibiotic group. Although not comparable to vancomycin, the L-PRP gel also exhibited antimicrobial efficacy in vivo. They suggested that a combination of L-PRP gel and antibiotics could be a favourable alternative for the treatment of osteomyelitis.

**Human clinical studies**

Several controversies exist in clinical studies, which make the conclusion hard. Different methods for PRP preparation and accordingly different concentration of platelets together with different qualitative assessments in different human populations alter the impact of these studies. The major effectiveness of PRP appears to be in the early stages of wound healing however, most of the clinical studies followed their patients for more than 6 months which make the comparison of their results with those of the animal studies, hard. Although the clinical studies suggested PRP as a treatment option and reported its effectiveness in the early stages of healing, however, their long-term results showed no significant differences with a control or placebo groups. It should be remembered that clinical studies use different populations that are different in many variables such as age, sex, personality, etc. However, in comparison between different clinical studies, some important points are indicated. Here, we discuss some of these researches. A brief report of the presented studies is provided in Table 3.

<table>
<thead>
<tr>
<th>No</th>
<th>Reference</th>
<th>Model of injury</th>
<th>Type of the study</th>
<th>Effective/non-effective/harmful</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>De Jonge et al.81</td>
<td>Chronic tendinopathy</td>
<td>Randomised control trial study</td>
<td>Non-effective</td>
</tr>
<tr>
<td>2</td>
<td>Finnoff et al.60</td>
<td>Chronic tendinopathy</td>
<td>Part A: retrospective observational study. Part B: prospective observational study</td>
<td>Effective</td>
</tr>
<tr>
<td>3</td>
<td>Jo et al.82</td>
<td>Rotator cuff tear</td>
<td>Level of 2 evidence of a cohort study</td>
<td>Non-effective</td>
</tr>
<tr>
<td>4</td>
<td>Rodeo et al.83</td>
<td>Rotator cuff tear</td>
<td>Level of 2 evidence of the randomised controlled trial study</td>
<td>Negative effect</td>
</tr>
<tr>
<td>5</td>
<td>Weber et al.84</td>
<td>Rotator cuff tear</td>
<td>Prospective, double-blinded, randomised study (level 1 of evidence)</td>
<td>Non-effective</td>
</tr>
<tr>
<td>6</td>
<td>Radice et al.65</td>
<td>ACL reconstruction</td>
<td>Prospective single-blinded study</td>
<td>Effective</td>
</tr>
<tr>
<td>7</td>
<td>Vogrin et al.66</td>
<td>ACL reconstruction</td>
<td>Clinical trial study</td>
<td>Effective</td>
</tr>
<tr>
<td>8</td>
<td>de Almeida et al.85</td>
<td>ACL reconstruction</td>
<td>Level 1 – evidence randomised controlled trial study</td>
<td>Non-effective</td>
</tr>
<tr>
<td>9</td>
<td>Magnussen et al.86</td>
<td>ACL reconstruction</td>
<td>Level III – retrospective comparative study</td>
<td>Non-effective</td>
</tr>
<tr>
<td>10</td>
<td>Filardo et al.87</td>
<td>Knee chondropathy or osteoarthritis</td>
<td>Randomised clinical trial</td>
<td>Non-effective</td>
</tr>
<tr>
<td>11</td>
<td>Halpern et al.88</td>
<td>Early osteoarthritis</td>
<td>Prospective cohort study</td>
<td>Non-effective</td>
</tr>
<tr>
<td>12</td>
<td>Patel et al.63</td>
<td>Early osteoarthritis</td>
<td>Randomised clinical trial</td>
<td>Effective</td>
</tr>
<tr>
<td>13</td>
<td>Ibrahim and Dowling64</td>
<td>Avascular necrosis</td>
<td>Case report</td>
<td>Effective</td>
</tr>
<tr>
<td>14</td>
<td>Döri et al.89</td>
<td>Deep intra-bony defects</td>
<td>Randomised blind clinical trial</td>
<td>Non-effective</td>
</tr>
<tr>
<td>15</td>
<td>Tatullo et al.85</td>
<td>Maxillary reconstruction</td>
<td>Randomised blind clinical trial</td>
<td>Effective</td>
</tr>
<tr>
<td>16</td>
<td>Bajaj et al.66</td>
<td>Mandibular degree II furcation defects</td>
<td>Randomised blind clinical trial</td>
<td>Effective</td>
</tr>
</tbody>
</table>

**Table 3. Effects of PRP or PRP gel in different human clinical studies.**

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)

**Tendinopathy**

Treatment of tendinopathy is one of the areas of debate. Several methodologies tried to introduce a novel approach in this regard. PRP is one of the treatment modalities that have been tested in clinically ill patients engaged with such injuries. De Jonge et al. studied the effects of PRP injection in patients with chronic mid-portion Achilles tendinopathy at 1-year follow-up. Fifty-four patients, aged 18–70 years, with chronic tendinopathy 2 to 7 cm proximal to the Achilles tendon insertion, were randomised to receive either a blinded injection containing PRP or saline (placebo group) in addition to an eccentric training program. Their results indicated that PRP showed no clinical and ultrasonographic superiority over a placebo injection. In another study, Finnoff et al. determined whether US-guided percutaneous needle tenotomy followed by a PRP injection would result in pain reduction, functional improvement or structural alterations in patients with chronic, recalcitrant tendinopathy. Their study was designed in two parts. Part A was a retrospective observational study. Part B was a prospective observational study. In part A, subjects completed a survey obtaining anthropomorphic, demographic, pain, and functional data. Subjects’ platelet, haemoglobin and WBC concentrations of their whole blood and PRP samples were also obtained. In part B, subjects returned to the clinic for a diagnostic US, which was compared with their pre-procedure diagnostic US. In this case series, Finnoff et al. concluded that the US-guided percutaneous needle tenotomy followed by PRP injection was a safe and effective treatment for chronic, recalcitrant tendinopathy, and this treatment was associated with ultra-sonographically apparent improvements in tendon morphology. A 76% of the subjects experienced a significant improvement in their pain, function improved significantly in 74% of subjects, with a mean improvement in the subjects’ function and worst pain scores of 68% and 58%, respectively. Furthermore, 83% of the subjects expressed satisfaction with their outcomes after the procedure and would recommend the procedure to a friend. It is interesting to note that more patients were satisfied with their outcomes (83%) than would be suggested by the percentage of patients who had significant improvements in their function and pain (68% and 58%, respectively). However, because of the intrinsic limitations of the study design and the heterogeneity of the treated tendons, further research is required to corroborate their findings. One of the major limitations of their study was that they only compared the symptoms and US results in the PRP-treated groups and did not compare the PRP-treated results with a control group. They also did not use PRP as the whole treatment modality and therefore the results of the study should be conservatively considered.

**Rotator cuff tears**

The prevalence of rotator cuff tendon injuries is high in human patients. The healing capability of the tendons even after simple tenotomy is low and therefore the outcome is poor. PRP has been tested in the patients with such injuries. Jo et al. tested the efficacy of PRP in the rotator cuff tendon healing in a level 2 evidence cohort study. Forty-two patients with full-thickness rotator cuff tears were included. The patients were informed about the use of PRP before surgery and decided themselves whether to have PRP placed at the time of surgery. Nineteen patients underwent arthroscopic rotator cuff repair with PRP and 23 without. PRP was prepared via platelet pheresis and applied in the form of a gel threaded to a suture and placed at the interface between tendon and bone. Their results indicated that the PRP-gel application to arthroscopic rotator cuff repairs did not accelerate recovery with respect to pain, range of motion, biomechanical strength, functional scores or overall satisfaction as compared with conventional repair up to 16-month follow up. As it has been discussed earlier, most of the insignificant comparisons were provided at long-term follow up in clinically tested studies and therefore it appears that the long-term follow up can impact on the conclusion of these studies.

In another study, Rodeo et al. evaluated the effects of PRF matrix (PRFM) on rotator cuff tendon healing in a level 2 evidence randomised controlled trial study. In their study, 79 patients undergoing arthroscopic rotator cuff tendon repair were randomised intra-operatively to either receive PRFM at the tendon-bone interface (n = 40) or standard repair with no PRFM (n = 39). They had clinically and ultrasonographically followed up their patients after the surgery for one year and indicated that the PRFM had no demonstrable effect on tendon healing, tendon vascularity, manual muscle strength or clinical rating scales. They suggested that PRFM might have a negative effect on healing. One of the major limitations of their study was that they did not count the platelet concentration of the PRFM. Actually they failed to report the information concerning the composition of the actual PRFM that each patient received (platelet number, presence of WBCs, cytokine content, etc.) and therefore, the results of that study are doubtful and should be conservatively included in the conclusion. Weber et al. also represented a prospective, double-blinded, randomised study (level 1 of evidence) to assess the usage of PRFM in rotator cuff surgery. Two groups of treated (PRFM) and control were selected (each with 30 patients). Their results indicated that the PRFM did not significantly improve perioperative morbidity, clinical outcomes (between 3 months to 1 year follow up) or structural integrity.

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)

ACL reconstruction

ACL injuries are the most prevalent ligament injuries in humans. The torn ACL is not repaired even after direct suturing and therefore replacement of the torn ligament is warranted especially in those complete sprain injuries. Based on the literature, it appears that PRP is an effective option in improving the graft healing after ACL reconstruction. Radice et al.84 determined whether usage of PRP-gel affects MRI findings in the ACL graft during the first year after reconstruction. A prospective, single-blinded study of 50 ACL reconstructions in 50 patients was performed. In group A (study group), PRP-gel was added to the graft with a standardised technique, and in group B (control group) no PRP-gel was added. An MRI study was performed post-operatively between 3 and 9 months in group A and between 3 and 12 months in group B. Their results indicated that ACL reconstruction by PRP-gel achieved complete homogenous grafts assessed by MRI, in 179 days compared with 369 days for ACL reconstructions without PRP-gel. This represented a time shortening of 48% with respect to ACL reconstruction without PRP-gel. In another study, Voigrin et al.82 determined whether the use of platelet gel accelerates early graft revascularisation after ACL reconstruction. The gel was produced from autologous PRP and applied locally. After 4–6 weeks, the PG-treated group demonstrated a significantly higher level of vascularisation in the osteoligamentous interface than the control group. They concluded that locally applied platelet gel enhanced early revascularisation of the graft in the osteoligamentous interface zone after ACL reconstruction.

de Almeida et al.85 in a level 1 evidence randomised, controlled trial study investigated the effect of PRP on the healing of the patellar tendon harvest site and the clinical outcome of the patients underwent ACL reconstruction with patellar tendon graft. They had two treated (PRP) and control (no treatment) groups. They concluded that PRP had a positive effect on patellar tendon harvest site healing on MRI after 6 months and also reduced pain in the immediate postoperative period. Questionnaire and isokinetic testing results were not different between the groups at 6 months86. The major merit of their study was that they served a control for their treatment group. Although they stated that the usage of PRP was beneficial, the outcome was not significantly better than those of the control ones after 6 months of surgery. Therefore, the results of the study were approximately similar to those of the previous ones and again, this should be highlighted as it appears that PRP is not effective in long-term outcome.

Magnussen et al.86 evaluated the effect of intra-operative PRP on patient-reported outcomes 2 years after ACL reconstruction with tibialis anterior allograft, in a level III retrospective comparative study. Fifty patients who underwent allo- graft ACL reconstruction with intra- operative application of PRP to the graft were matched with 50 allograft ACL reconstructions without PRP use. Their study demonstrated that, although the PRP application in tibialis allograft ACL reconstructions appeared safe, its clinical benefit was minor and short term. No differences in patient-reported outcomes or number of additional surgeries at 2 years were noted.

Effects of PRP on hard tissue (bone and cartilage)

There are several controversies with regard to the efficacy of PRP on hard tissues, in clinical studies. However, similar to soft tissues, it appears that almost comparable results are supported by those hard tissue-based studies, which means that PRP is generally short acting and cannot alter the long-term outcome. However, some exceptions exist. Filardo et al.87 showed the efficacy of PRP in a randomised double-blind prospective trial, by comparing PRP to hyaluronic acid injections for the treatment of knee chondropathy or OA. They indicated that PRP injections offer a significant clinical improvement up to 1 year of follow up. However, PRP results were not better than those obtained with hyaluronic acid injections, and thus it should not be considered as first line treatment. In another study, Halpern et al.88 investigated whether PRP therapy for early knee OA is associated with good clinical outcomes and a change in MRI structural appearances. The design was a prospective cohort study that followed patients 1 year after PRP therapy for knee OA. Their results indicated that PRP had no significant role in clinical and MRI outcomes of the patients. Patel et al.63, in a level 1 evidence randomised controlled trial also studied the role of PRP on symptomatic relief in early OA of the knee. A total of 78 patients (156 knees) with bilateral OA were divided randomly into three groups. Group A (52 knees) received a single injection of PRP, group B (50 knees) received 2 injections of PRP 3 weeks apart, and group C (46 knees) received a single injection of normal saline. WBC-filtered PRP, with a platelet count three times that of baseline (PRP type 4B), was administered in all. The clinical outcome was evaluated using the Western Ontario and McMaster Universities Arthritis Index questionnaire before treatment and at 6 weeks, 3 months and 6 months after treatment. They also evaluated the pain by a visual analogue scale, and overall satisfaction with the procedure and complications were noted. Their results indicated that a single dose of WBC-filtered PRP in concentrations of 10 times higher than normal is as effective as two injections to alleviate symptoms in early knee OA. The results, however, were not long lasting and were not significant after 6 months. Both the groups treated with PRP had better results than
did the group injected with saline only. Their results confirmed that the higher concentration of PRP was more effective than the lower one. This supports the results of the animal studies and this phenomenon that the main mechanism of PRP in healing is its immunogenic activity and its potential to induce inflammation. Perhaps a higher platelet concentration can increase the amount of inflammation at injured site.

Avascular necrosis is a progressive condition characterised by bone tissue cell death as a result of ischaemia, which is most often observed in weight-bearing joints. The traditional treatment of this disease process in the hip includes surgical decompression and joint replacement. Ibrahim and Dowling reported a novel non-surgical approach for treating advanced-stage degenerative avascular necrosis of the hip by using autologous PRP. They stated that the patient had significant functional improvements after this intervention without needing further treatment except for physical therapy. However, their study was a case report and could not be included in the conclusion. In another study, Döri et al. reported that application of PRP did not result in an improvement in the deep intra-bony defects after 5 years in humans. This study supports our hypothesis that PRP is not effective in long-term follow up.

PRP has been frequently used in maxillofacial surgery. Clinical and histological examination of Tatullo et al. revealed the potential use of PRF, associated with deproteinised bovine bone, as grafting materials in pre-implantology sinus grafting of severe maxillary atrophy, in comparison with a control group, in which only deproteinised bovine bone was used as reconstructive material. They indicated that using PRF reduced the healing time, with favouring optimal bone regeneration. However, their investigation was a short-term study but their results were encouraging.

Bajaj et al. explored the clinical and radiographical effectiveness of autologous PRF and autologous PRP in the treatment of mandibular degree II furcation defects in subjects with chronic periodontitis. Seventy-two mandibular degree II furcation defects were treated with either autologous PRF with open flap debridement (OFD; 24 defects) or autologous PRP with OFD (25), or OFD alone (23). All clinical and radiographic parameters showed statistically significant improvement at both the test sites (PRF with OFD and PRP with OFD) compared with those with OFD alone. Relative vertical clinical attachment level gain was also greater in PRF and PRP sites as compared with control site, and relative horizontal clinical attachment level gain was statistically significantly greater in both PRF and PRP than in the control group. They concluded that the usage of autologous PRF or PRP were both effective in the treatment of furcation defects with uneventful healing of sites. It appears PRP is more effective in maxillofacial injuries than other musculoskeletal injuries (e.g. Achilles tendon rupture, OA).

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

This review showed that more than 90% of the in vitro and in vivo studies suggested that application of PRP stimulates the healing process. In addition, more than 50% of the human clinical studies suggested that PRP is not effective in the healing response of the injured tissues. Based on the presented results of this review, there are many factors that have a role in this regard. We showed that most of the in vivo animal studies used an allogous form of PRP while the clinical human studies used an autogenous form of PRP. The mechanism of action of PRP on wound healing has been discussed in this review. Possibly the effects of allogous PRP on inflammation is one of the factors that explain why in vivo application of PRP is effective in promoting the healing response. It is well accepted that the autologous form of PRP is less immunogenic, thus it is theoretically possible that the autologous PRP initiated the inflammation less than that of the allogous form, which is important in wound healing. We have previously discussed the importance of inflammation in wound healing.

Second, most of the human clinical studies used different methodologies to assess the efficacy of PRP in the healing of soft and hard connective tissues. This may affect the clinical results and therefore, make their conclusion hard to follow. Unlike human clinical studies, the in vivo animal studies used more standard methodologies that are straightforward. For example, histological and biomechanical analyses are well-accepted methods in defining the significant differences between the treated and control groups. However, the clinical methodologies such as imaging techniques or clinical examinations such as palpations, history taking use different scoring criteria and therefore it is hard to compare the results of these types of studies. Different populations are also compared in clinical studies, thus several variables exist that affect the outcome of the studies. For example, age, sex, personality and other factors are different between the test and control populations. The follow-up methods in the human patients are also different. Moreover, in many clinical studies, the researchers, inform their patients about the treatment modality. Knowing the super...
trials. Proponents of PRP therapy medicine has produced ‘promising’ use of PRP for nerve injury and sports clinical trials. For example, clinical confirmed in large-scale controlled preclinical trials have not yet been The results of basic science and evaluation of the effectiveness of this approach in humans in treatment of musculoskeletal injuries. Approach to suggest the PRP as an effective priority of the treatment modality affects patient mentality. This can be effective in reducing symptoms and improving the outcome of the disease in these patients compared to those of the disoriented patients (placebo effect). Unlike human clinical studies, the in vivo animal studies are more standard, and less effective variables exist. For example, in such studies, one genus of animals is selected. All animals are selected in the same sex and age and the nature of the experimentally induced injury is also the same. One person does the treatment. All of the animals are kept in the same place and condition. Their feeding timetable is also the same. These variables may explain why there are controversies between the in vivo animal studies with those of the human clinical studies. As a final reason, presence of various preparation methods and different platelet concentrations obtained, as well as other variables, such as activation modalities and presence of WBCs within the platelet concentrate and many other aspects, are confusing factors when comparing the results obtained in different studies and complicate the research in this field, both in preclinical studies and in evaluation of the effectiveness of this approach in humans in treatment of musculoskeletal injuries. Usage and clinical validation of PRP is still in the early stages. The results of basic science and preclinical trials have not yet been confirmed in large-scale controlled clinical trials. For example, clinical use of PRP for nerve injury and sports medicine has produced ‘promising’ but ‘inconsistent’ results in early trials. Proponents of PRP therapy argue that negative clinical results are associated with poor-quality PRP produced by inadequate devices. The fact that most gathering devices capture a percentage of a given thrombocyte count is a bias, since there is significant inter-individual variability in the platelet concentration of human plasma. More is not necessarily better in this case. The variability in platelet concentrating techniques may alter platelet degranulation characteristics that could affect clinical outcomes.

Conclusion

It appears that PRP has some beneficial effect especially at pre-clinical level. Most of the in vitro and in vivo animal studies used allogous methods of preparation, concentration, activation and form (gel vs. solution), the results were encouraging and confirmed the efficacy of PRP in soft and hard connective tissue healing in different animal species. However, at clinical level, there are many controversies. In these studies, where the autogenous PRP is normally used, in most instances, no significant effect of PRP has been reported on tissue healing. In general, PRP is an effective conservative treatment without the complications and cost of surgery, and with a moderately high rate of success of resolving various musculoskeletal conditions; however, it should not be placed in the first line of treatment modality because it is almost effective in short term period. Future clinical trial studies should be designed in order to test the real efficacy of PRP in a more standard manner and it appears it is still soon to suggest the PRP as an effective treatment modality in the field of regenerative medicine and orthopaedic surgery especially at clinical level.

Abbreviations list

ACL, anterior cruciate ligament; ADSC, adipose tissue-derived stem cell; APD, acellular porcine dermal patch; BMP, bone morphogenic protein; CTGF, connective tissue growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2; IL-8, interleukin-8; KGF, keratinocyte growth factor; OA, osteoarthritis; PDGF, platelet-derived growth factor; PPF, platelet-poor fibrin; PPP, platelet-poor plasma; PRCR, PRP-clot releasate; PRF, platelet-rich fibrin; PRFM, PRF membrane; PRP, platelet-rich plasma; PRPFM, PRP fibrin matrix; MRI, magnetic resonance imaging; MRSA, methicillin-resistant Staphylococcus aureus; RBCs, red blood cell; TA, trimacinolone acetone; TGF-β, transforming growth factor-β; TKR, tyrosine kinase receptor; TSC, tendon stem cell; US, ultrasound; VEGF, vascular endothelial growth factor; WBCs, white blood cell.

References

8. Oryan A. and Shoushtari A.H. biomechanical properties and dry weight content of the developing superficial
74. Oryan A, Shoushtari AH. Histology and ultrastructure of the developing superfi-


Review