■ REVIEW ARTICLE

The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery

A REVIEW OF THE LITERATURE

J. Alsousou,
M. Thompson,
P. Hulley,
A. Noble,
K. Willett

From The University of Oxford, Oxford, England

Although mechanical stabilisation has been a hallmark of orthopaedic surgical management, orthobiologics are now playing an increasing role. Platelet-rich plasma (PRP) is a volume of plasma fraction of autologous blood having platelet concentrations above baseline. The platelet α granules are rich in growth factors that play an essential role in tissue healing, such as transforming growth factor-β, vascular endothelial growth factor, and platelet-derived growth factor. PRP is used in various surgical fields to enhance bone and soft-tissue healing by placing supraphysiological concentrations of autologous platelets at the site of tissue damage. The easily obtainable PRP and its possible beneficial outcome hold promise for new regenerative treatment approaches.

The aim of this literature review was to describe the bioactivities of PRP, to elucidate the different techniques for PRP preparation, to review animal and human studies, to evaluate the evidence regarding the use of PRP in trauma and orthopaedic surgery, to clarify risks, and to provide guidance for future research.

■ J. Alsousou, MRCS, Clinical Research Fellow
■ K. Willett, FRCS, Professor of Trauma Orthopaedic Surgery
Kadoorie Trauma Research Unit
Nuffield Department of Orthopaedic Rheumatology and Musculoskeletal Sciences, University of Oxford, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK.

■ M. Thompson, PhD, University Lecturer in Orthopaedic Biomechanics
■ P. Hulley, PhD, Research Fellow in Orthopaedic Science
The Botnar Research Centre
Institute of Musculoskeletal Sciences
Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Windmill Road, Oxford OX3 7LD, UK.

■ A. Noble, PhD, Professor of Engineering Science
Department of Engineering Science
Institute of Biomedical Engineering, Old Road Campus Research Building, University of Oxford, Headington, Oxford OX3 7DQ, UK.

Correspondence should be sent to Mr J. Alsousou; e-mail: josephalsousou@doctors.org.uk

doi:10.1302/0301-620X.91B8.22546 $2.00

J Bone Joint Surg [Br] 2009;91-B:987-96.
Received 5 March 2009; Accepted 21 April 2009

The use of platelet-rich plasma (PRP) in tissue regeneration is a developing area for clinicians and researchers and has been employed in various fields of surgery. Although the growth factors and mechanisms involved are still poorly understood, the easy application of PRP in clinical practice and its possible beneficial outcome, including bone regeneration, reduction of bleeding and rapid tissue healing, hold promise.[1] An important feature of PRP is that this autologous product eliminates concerns about immunogenic reactions and disease transmission.

Allogeneic fibrin glue was originally described in 1970[2] and is formed by polymerising fibrinogen with thrombin and calcium. In 1990, Gibble and Ness[3] introduced autologous fibrin gel (fibrin sealant or fibrin glue), a biomaterial with haemostatic and adhesive properties. The addition of platelets, as a rich source of bioactive factors, came later[4,5] This shifted the focus of research away from recombinant human growth factors, which have a short life span and inefficient local delivery to target cells. Recombinant factors are expensive, and high doses may be required to achieve any therapeutic effect. Autologous platelet concentrates, however, offer an easy, cost-effective way to obtain high concentrations of growth factors for tissue healing and regeneration. The different bioactive factors, which are released upon platelet activation, include platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and epidermal growth factor (EGF). These proteins are histopromotive factors. They set the stage for tissue healing which includes cellular chemotaxis, proliferation and differentiation, removal of tissue debris, angiogenesis, and the laying down of extracellular matrix.[4,6]

The clinical use of PRP for a wide variety of applications has been described, particularly in periodontal, craniofacial and spinal surgery. Collectively, these studies provide strong evidence to support the clinical use of PRP, but many are anecdotal and few include controls to definitively define the role of PRP. There is little consensus regarding the production and characterisation of PRP, which has precluded the establishment of the standards necessary to integrate the extensive relevant literature in basic and clinical science. This article reviews the published information on the role of PRP in trauma and orthopaedic surgery, deals with the different techniques available for its preparation, and clarifies possible risks related to its use.

Methods

We searched several databases, including EMBASE,[7] CINAHL[8] and MEDLINE,[9] as well as reference lists of articles and contacted their authors. Keywords used included platelet-rich plasma, platelet concentrates, platelet gel, PRP preparation, PRP and trauma, PRP and bone, and PRP and tendon. Only articles published in peer-reviewed journals that met the following criteria were assessed:

(i) *In vitro* studies which had reported on the effect of PRP on cultures of primary cell lines;

(ii) *In vivo* animal studies evaluating the effect of PRP on regeneration of hard or soft tissue;

(iii) Clinical articles on treatment with PRP;

(iv) Outcome studies.

The criteria were applied to titles and abstracts, and potentially eligible studies were chosen for retrieval. The reference lists of all eligible articles were reviewed for additional relevant papers.

The biological properties of PRP. Platelet-rich plasma (PRP), also known as platelet-enriched plasma (PeRP), platelet-rich concentrate (PRC), autogenous platelet gel, or platelet releasate, may be defined as the volume of the plasma fraction of autologous blood having a platelet concentration above baseline.[10] A concentration of 1 407 640 µl (SD 320 100) of platelets in plasma has been suggested to be the working definition of PRP.[11] This is a platelet count five times higher than that of the blood, which is normally 150 000 µl to 350 000/µl, with an approximate mean of 200 000/µl. PRP refers to autologous preparations. However, a preparation of allogenic PRP (aPRP) may serve as an alternative in the case of patients who refuse to be subjected to a blood drawing procedure or are not suitable for autologous PRP.[12]

Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cell, and are formed in the marrow. They are the smallest of the blood cells, round or oval in shape and approximately 2 µm in diameter. They lack nuclei but contain organelles and structures such as mitochondria, microtubules, and granules (α, δ, λ). The α granules, bound by a membrane, are formed during maturation of the megakaryocytes. They are about 200 nm to 500 nm in diameter, and number approximately 50 to 80 per formed platelet.[13] They contain more than 30 bioactive proteins, many of which have a fundamental role in haemostasis or tissue healing. Haemostasis can be considered to be the first stage of healing.[14,15]

The properties of PRP are based on the production and release of multiple growth and differentiation factors when the platelets are activated. Platelets begin actively secreting these proteins within ten minutes of clotting,[16] with more than 95% of the pre-synthesised growth factors secreted within one hour.[17] After the initial burst of growth factors, the platelets synthesise and secrete additional such factors for the remaining several days of their life span.[17] However, the factors that determine the biological properties of PRP are unknown, and there is insufficient understanding of the clinical effects of its application.[10] The combined action of all these growth factors is complex, and each may have a different effect on a particular tissue. Growth factors may also interact with each other, activating different sets of signalling pathways. Different isoforms of growth factors have varying effects that may enhance or inhibit osseous and soft-tissue repair, depending on the mode of release of the factor and the dynamics of the wound environment.[15]

Biological activity of PRP. Healing of both soft and hard tissue is mediated by a complex array of intra- and extracellular events that are regulated by signalling proteins. This process is incompletely understood. Disruption of the vascular structure as a result of injury leads to the formation of fibrin and platelet aggregation. A stable blood clot is then formed by coagulation of the blood. Subsequently, several growth factors are released into the injured tissue from the platelets and other cells that induce and support healing and tissue formation.[15,16] PRP is also activated by the addition of thrombin and calcium, resulting in the release of a cascade of growth factors from the α granules[17] (Fig. 1). These granules contain numerous proteins that provide a powerful influence on tissue healing. They include platelet-derived growth factor (PDGF-αα, ββ and αβ isomers), transforming growth factor (TGF-β, β1 and β2 isomers), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), VEGF, epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin-1.[4,10,13,15,16] Collectively, these proteins are members of the families of growth factors, cytokines and chemokines. The interaction between these growth factors and surface receptors on the target cells activates the intracellular signalling pathways that induce the production of proteins needed for the regenerative processes such as cellular proliferation, matrix formation, osteoid production and collagen synthesis.[18]

PRP also contains proteins such as fibrin, fibronectin, vitronectin and thrombospondin, which are known to act as cell adhesion molecules, important for the migration of osteoblasts, fibroblasts and epithelial cells.[18] Adult mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells express the cell membrane receptors that are specific to the growth factors included in PRP.[5,14] It is therefore suggested that the growth factors included in the platelet concentrates activate several cell types involved in tissue healing, and thus promote healing of soft tissue and regeneration of bone.

Growth factors in PRP. The levels of growth factors released from the platelets upon activation are commonly quantified by enzyme-linked immunosorbent assay (ELISA).[19-21]

PDGF was first found in platelets, especially in the α granules.[22] It is also found in other cells, such as macrophages,[23] endothelial cells,[24] monocytes and fibroblasts, and in the bone matrix.[25] Three isoforms exist: αα, ββ and αβ isomers.[26] The reason for three distinct forms remains

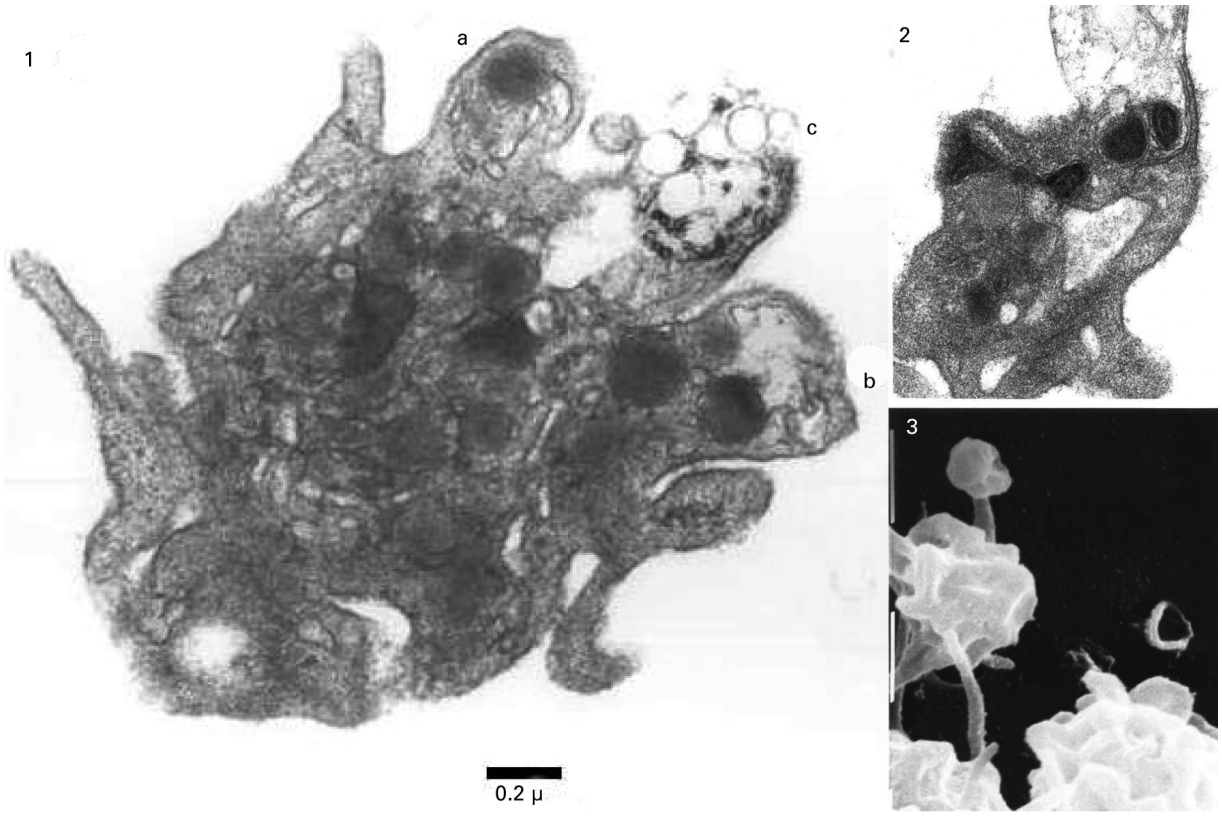

Fig. 1

On activation, granules appear to migrate to the periphery of the platelet and leave through platelet plasma membrane blebs, (a) protrusions, (b) rupture of the limiting membrane with loss of electron density (c). 2) Enlarged section shows blebbing of the α granule. 3) SEM picture of an activated platelet shows a multivesicular bleb at the tip of a pseudopod with another remnant of an α granule lying on the filter[16] (reprinted from Thrombosis Research 45, Polasek J, Richardson M, Moore MA, Blajchman MA. Evidence for an alternative mechanism of human platelet secretion involving peripheralization of secretory granules and formation of membrane-assisted multivesicular structures). Copyright 1987, with permission from Elsevier.

unclear, but differential binding by various receptor cells such as endothelium, fibroblasts, macrophages and marrow stem cells has been suggested.[25] The most important specific activities of PDGF include angiogenesis and macrophage activation,[27] proliferative activity on fibroblasts,[28] chemotaxis for fibroblasts and collagen synthesis.[29] It also enhances the proliferation of bone cells.[30] Using a freeze-thaw cycle to release proteins, Weibrich et al[11] measured the levels of PDGF-αβ and PDGF-ββ in specimens of PRP from 115 patients. The minimum and maximum values for each typically spanned one to two orders of magnitude, with means and SD of 117.5 ng/ml (SD 63.4) and 9.9 ng/ml (SD 7.5), respectively. There was little or no correlation between the levels of these individual proteins, donor age or gender.

TGF-β has been referred to as a member of a superfamily of growth and differentiation factors, including the bone morphogenetic proteins.[31] TGF-β has three different isoforms: β1, β2 and β3.[32] TGF-β has been observed to promote the production of extracellular matrix,[33] to enhance the proliferative activity of fibroblasts,[28] to stimulate biosynthesis of type I collagen and fibronectin and to induce deposition of bone matrix.[34] TGF-β may inhibit osteoclast formation and bone resorption, thus favouring bone formation over resorption.[35] The level of TGF-β1 in PRP is 169.4 ng/ml (SD 84.5) and that of TGF-β2 is 0.4 ng/ml (SD 0.3), according to Weibrich et al.[11]

IGF-I is chemotactic for fibroblasts and stimulates protein synthesis.[36] It enhances bone formation by the proliferation and differentiation of osteoblasts.[36-38] Its concentration in PRP is reported to be 84.2 ng/ml (SD 23.6).[11]

PDEGF was discovered in 1962.[39] It stimulates epidermal regeneration, promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts, and enhances the production and effects of other growth factors.[6]

PDAF has the capacity to induce vascularisation by stimulating vascular endothelial cells.[40] Several cytokines and growth factors, including IGF-1, TGF-α, TGF-β, PDGF, PDEGF, and interleukin-1b upregulate PDAF.

PF-4 is also released from the α granules of platelets and may be partially responsible for the initial influx of neutrophils into wounds. It also acts as a chemoattractant for fibroblasts and promotes blood coagulation by moderating the effects of heparin-like molecules.[41]

Table I. The effect of the growth factors produced by platelets, and their average concentrations in platelet-rich plasma (PRP)

Growth factor*	Effect	PRP concentration (SD)
PDGF	Macrophage activation and angiogenesis Fibroblast chemotaxis and proliferative activity Enhances collagen synthesis Enhances the proliferation of bone cells	αβ 117.5 ng/ml (63.4) ββ 9.9 ng/ml (7.5)
TGF-β	Enhances the proliferative activity of fibroblasts Stimulates biosynthesis of type I collagen and fibronectin Induces deposition of bone matrix Inhibits osteoclast formation and bone resorption	β1: 169.9 ng/ml (84.5) β2: 0.4 ng/ml (0.3)
IGF-I	Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation by proliferation and differentiation of osteoblasts	84.2 ng/ml (23.6)
PDEGF	Promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts	470 pg/ml (320)
PDAF	Induces vascularisation by stimulating vascular endothelial cells	
PF-4	Stimulates the initial influx of neutrophils into wounds A chemoattractant for fibroblasts A potent antiheparin agent	0.189 nmol/ml (0.07)
EGF	Cellular proliferation Differentiation of epithelial cells	51 pmol/l (5)
VEGF	Angiogenesis Migration and mitosis of endothelial cells Creation of blood vessel lumen Creates fenestrations Chemotactic for macrophages and granulocytes Vasodilation (indirectly by release of nitrous oxide)	76 to 854 pg/ml

* PDGF, platelet-derived growth factor; TGF, transforming growth factor; IGF, insulin-like growth factor; PDEGF, platelet-derived endothelial growth factor; PDAF, platelet-derived angiogenesis factor; PF-4, platelet factor 4; EGF, endothelial growth factor; VEGF, vascular endothelial growth factor

VEGF is a subfamily of a platelet-derived growth factor family of cystine-knot growth factors. They are important signalling proteins involved in both vasculogenesis and angiogensis, which paves the way for healing.[42] *In vitro*, VEGF-A has been shown to stimulate the mitogenesis of endothelial cells and cell migration. It is also a vasodilator and increases microvascular permeability.

EGF acts by binding with high affinity to the epidermal growth factor receptor (EGFR) on the cell surface, triggering an increase in the expression of certain genes that ultimately lead to DNA synthesis and cell proliferation.[43]

The effect of platelet concentration on the efficiency of PRP. The effect of PRP on tissue healing is a function of many variables, including platelet concentration, the volume of PRP delivered, the extent and type of injury and the overall medical condition of the patient. A concentration of 1 407 640 µl (SD 320 100) or (10% of the PRP volume) plasma has been suggested to be the working definition of PRP.[31,44] However, Anitua et al[14] stated that the platelet count of PRP should be just above 300 000 µl. On the other hand, Choi et al[45] showed that high PRP concentrations (> 10%) suppressed, but that low PRP concentrations (1% to 5%) stimulated, the viability and proliferation of alveolar bone cells. Thereby, supporting the view that variations in PRP concentrations may lead to variable effect on tissue regeneration. The effect of growth factors produced by platelets is summarised in Table I.

The concentration of PRP is correlated with the platelet count in the whole blood from which it is derived and is related to the gender of the donor although there is no effect of age.[11] The concentration of growth factors also increases with an increasing number of platelets.[20]

The method used to measure platelet concentration can affect the result. The manual counts will record individual platelets, whereas automated scanning, such as by the Coulter Counter, may count clumps of platelets as a single platelet thereby underestimating their number.[46] This highlights the importance of using consistent methods and properly interpreting the results of other investigators, whose methods of counting may differ. Weibrich and Kleis[47] have suggested that different individuals may require different platelet concentration ratios to achieve a comparable biological effect.

Preparation of PRP. This can be prepared in a laboratory, an operating theatre or a clinic from blood collected in the immediate period before treatment. A small amount of autologous PRP can be obtained in minutes. There are three techniques for preparation: the gravitational platelet sequestration (GPS) technique, standard cell separators, and autologous selective filtration technology (plateletpheresis).

The GPS is a table-top centrifuge system. When anticoagulated blood is centrifuged, three layers become evident. The bottom layer is comprised of red blood cells (specific gravity = 1.09), the middle of platelets and white blood cells (buffy coat, specific gravity = 1.06), and the top of plasma (specific gravity = 1.03). The PRP yield is approximately 10% of the volume of whole blood drawn. This system used a flat-bottomed, 60-ml plastic centrifuge tube. The PRP volume of about 5 ml can be collected following a 12-minute spin at 3200 rpm. With this device, the red blood cells cannot be collected separately and are therefore discarded.

Standard cell separators and salvage devices generally operate on a full unit of blood.[48] In general, they use a continuous-flow centrifuge bowl or a continuous-flow disk separation technique and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations from two to four times baseline.[45] Weibrich and Kleis[47] described a discontinuous technique with a cell separator that produces a fivefold increase in platelet count. The red blood cells and some, or all, of the platelet-poor plasma (PPP) can be returned to the patient to maintain circulating volume.[44]

Small compact office systems have been developed that produce approximately 6 ml of PRP from 45 ml to 60 ml of blood, obviating the need for reinfusion.[47,49] These systems differ widely in their ability to collect and concentrate platelets, collecting from 30% to 85% of the available platelets and increasing the platelet concentration between two- and eightfold.[48,49] Some of the units permit the processing of two sets of disposables at once, or performing multiple sequential processes using the same disposable set, so that multiples of the 6 ml standard volume of PRP can be produced.

Centrifugation must be sterile and precisely suited to separating platelets from red blood cells with adequate concentrations of platelets.[48] Not all currently available commercial devices may be the same, and some probably do not concentrate active platelets in sufficient numbers to enhance healing. This might explain the variability of the clinical efficacy of PRP. Studies suggesting that there is no benefit from PRP might be based on a product of poor quality produced by inadequate devices.[5] Several studies suggest different centrifugation cycles in terms of time and force.[5] The centrifugation force may be a critical step in preparation of PRP as applied mechanical forces may damage platelets, thereby losing the granular load of the growth factors. One study evaluated the effect of different centrifugal forces and showed that spins > 800 *g* may reduce the amount of TGF-β released by the PRP.[50]

Selective filtration technology or plateletpheresis depends on a single-use disposable proprietary filter designed to concentrate platelets from whole blood. The platelets are captured on the filter and are then harvested to provide a platelet-rich concentrate (PRC) without the need for centrifugation. Compared to a commercial centrifuge-based method, the filtration device produces a blood fraction similarly enriched in platelets and growth factors.[51]

In general, most systems do not concentrate the plasma proteins of the coagulation cascade.[48] Plasma protein concentrations above baseline can be achieved through secondary ultrafiltration. The Autologous Growth Factor Filter (AGF) is a microporous, hollow cellulose fibre device with a volume of 8 ml which uses as a baseline produce PRP that has been manufactured by the cell separator device. It filters water after multiple passes of PRP through the filter. As much as two-thirds of the aqueous phase is removed by filtration, and thus the concentrations of the retained plasma proteins and formed elements are correspondingly increased.[52]

There are several choices of anticoagulant that can be used during preparation of PRP. Anticoagulant citrate dextrose-A (ACD-A) works well, as the citrate binds calcium and prevents coagulation, whereas the dextrose and other ingredients support platelet metabolism and viability. Citrate phosphate dextrose is similar to ACD-A but has fewer supportive ingredients and may therefore be less effective at maintaining platelet viability.[12] The use of ethylenediaminetetra-acetic acid (EDTA) is potentially more harmful in the preparation of PRP, and a large number of damaged platelets have been observed. Trisodium citrate solution is an anticoagulant with no negative effects on PRP preparation,[53] and consequently, ACD is the preferred anticoagulant.

Handling and application of PRP. Once the PRP is prepared it is stable in the anticoagulated state for eight hours or longer, permitting the blood to be drawn before operation and used as needed during lengthy procedures.[10] It must be activated for the platelets to release the contents of their α granules, with the clot that forms providing a vehicle to contain the secreted proteins and maintain their presence at the site of application. This is most commonly accomplished by adding a solution of 1000 units of topical bovine thrombin per millilitre of 10% calcium chloride to the PRP.[53] Marx[17] described a technique in which 6 ml of PRP, 1 ml of the calcium chloride/thrombin mix and 1 ml of air are introduced into a 10 ml syringe, with the air acting as a mixing bubble. The syringe is agitated for six to ten seconds to initiate clotting, and the clot then delivered. Man, Plosker and Winland-Brown[54] described an alternative technique for delivering the activated PRP. The PRP and calcium chloride/thrombin solution are mixed in a 10:1 ratio using a dual syringe system. The PRP is drawn into a 10 ml syringe and the activating solution is drawn into a 1 ml syringe. Both syringe plungers are connected to move in concert with both output ports connected to a dual spray applicator tip which allows both solutions to be mixed as they are applied to the

surgical bed. Because the α granules quickly release their contents on activation, Marx[17] states that the clotted PRP should be used within ten minutes of clot initiation. This is not a problem with the dual syringe delivery because the PRP is delivered to the wound site immediately after activation. In the case of other mixing techniques, it is important to transfer the clot to the surgical site before retraction, otherwise the clot that is transferred may be deficient in the secretory proteins. Most commercially available preparation systems have a PRP delivery technique similar to that of Man et al.[54]

In vitro and animal studies of PRP. Although platelet concentrates have been used to promote bone healing, the underlying mechanisms at cellular level remain poorly understood. The effect of PRP on bone cells may not be due to the action of a single growth factor but to the synergistic effects of the many such factors derived from platelets. The addition of PDGF or TGF to cell cultures does not induce enhanced cell proliferation such as that observed in the presence of a platelet concentrate.[55] Soffer et al[56] investigated the effects of human platelet lysate, consisting of damaged platelets with microparticles of cytoplasmic and cell membrane and soluble growth factors, on the bone cells of rat calvaria. Short-term exposure (≤ 24 hours) to platelet lysate promoted the proliferative and chemotactic functions of the bone cells, whereas long-term exposure had a negative effect, resulting in a decrease in alkaline phosphatase activity and of mineral formation. Another *in vitro* study also showed that PRP stimulated cell growth and differentiation of the cells of rat bone marrow for up to eight days.[19]

In vitro studies have confirmed the strong inductive properties of PRP. Lucarelli et al[57] estimated the effect of PRP on the proliferation of human stem cells and markedly increased cell numbers with an increase in concentration of PRP from 1% to 10%. Studies of lysed platelet solutions showed that they similarly stimulated the proliferation of human embryonic cells[58] and had a mitogenic effect on bone cells derived from human trabecular and adult rat bone marrow.[59] Thus, platelet-related activity may not be restricted to intact platelets, because micro-particles of cytoplasmic and cell membrane produced from activated platelets may also stimulate human bone cell proliferation. These findings conflict with the studies of Marx,[16,17] who considered that platelets damaged or rendered non-viable by PRP processing may not secrete bioactive growth factors, resulting in disappointing outcomes.

The effect of PRP on regeneration has been studied on damaged tendon modules.[60-63] All three TGF-β isoforms significantly increased type I and III collagen production in tendon fibroblasts.[60] Tendons cultured in 100% PRP showed enhanced gene expression of the matrix molecules, with no concomitant increase in the catabolic molecules.[61] Moreover, releases from both PRP and PPP clots stimulate tendon cell proliferation, in contrast to unclotted PPP.[62] In studies of human tenocyte culture both PRP, and PPP, stimulate cell proliferation and total collagen production. PRP but not PPP slightly increases the expression of matrix-degrading enzymes and endogenous growth factors.[63]

Although studies of primary cell culture provide the best platform for elucidating the effects of PRP at a molecular level, they provide a reductionist paradigm and data should be interpreted within that context.[58]

Several animal studies have provided encouraging results supporting the beneficial effect of PRP on bone healing. The use of PRP has been observed to improve bone healing in defects in the rabbit calvaria[59,64] and to facilitate the incorporation of particulate cancellous bone grafts in mandibular reconstructions in goats.[65]

According to Tomoyasu et al,[66] PRP and its soluble fraction stimulated osteoblastic differentiation of myoblasts and osteoblastic cells in the presence of BMP-2, BMP-4, BMP-6 and BMP-7, suggesting that platelets contain not only growth factors for proliferation but also novel potentiator(s) for BMP-dependent osteoblastic differentiation. In an animal model, Torres et al[67] found that the local application of PRP in bone defects enhances healing significantly at four weeks.

The positive effect of PRP on tendon healing has been established in several studies. In an *in vitro* study, Aspenberg and Virchenko[68] injected PRP percutaneously into transected tendo Achillis in the rat. This increased the strength and stiffness of tendon callus by about 30% after one week. Mechanical testing indicated an improvement in maturation of the callus. Kajikawa et al[69] showed that PRP injected locally in the rat patellar tendon increased the activation of circulation-derived cells and the immunoreactivity for types I and III collagen at an early phase of tendon healing. The osteoinductive effect of PRP on tendon-to-bone healing was evaluated on repair of the infraspinatus in a sheep model using MRI and histological study.[70] The results showed increased formation of new bone and fibrocartilage at the healing site.

However, others have concluded that there was little or no benefit from PRP. In an animal study, Aghaloo, Moy and Freymiller[71] grafted defects in the calvarium in rabbits with autogenous bone, PRP alone, or autogenous bone and PRP. The controls were not treated. Histomorphometric evaluation showed a tendency for slightly more bone formation when PRP was combined with autogenous bone than with bone alone, but this difference was not significant. A study by Chaput et al[72] suggested that PRP is not a major contributing factor to bone ingrowth at the bone-implant interface in the distal femur of rabbits.

There is some evidence that PRP has a limited or even a negative effect in certain delivery media.[35] PDGF was shown to inhibit intramuscular osteoinduction and chondrogenesis by demineralised bone matrix in immunocompromised mice.[73] In a similar model by the same author, PRP reduced the osteoinductivity of demineralised bone matrix implanted in immunocompromised mice, and the activities of both demineralised bone matrix and PRP were donor dependent.[74]

This analysis of the animal studies outlines the controversial nature of the use of PRP and the need to establish its clinical efficacy.

Clinical studies of PRP. In clinical studies, details of the quantity of PRP used and the methods of application are procedure specific. Although the majority of these studies have yielded excellent outcomes, most are only limited case studies or small series. This evidence of enhancement of tissue healing by PRP remains largely anecdotal. A small collection of clinical studies with prospective or retrospective controls have demonstrated a significant enhancement of healing of hard and soft tissue with the use of PRP.

One of the first clinical applications was the addition of autologous fibrin adhesive to cancellous bone during mandibular reconstruction. This study, published in 1994, described radiographic consolidation of bone after four weeks, as opposed to eight in controls which was attributed to enhanced osteoconduction given to the osteocompetent cells in the graft by the fibrin network developed by the concentrated platelets.[75]

When the PRP is activated, its advocates suggest that the benefits include restoration of bone and soft tissue, improved wound healing and a reduction in post-operative infection, pain and blood loss.[76] There have been numerous publications on the use of PRP for clinical applications in periodontal and oral surgery, maxillofacial surgery, plastic surgery with face lifts and cosmetic dermal fat grafts, spinal fusion by enhancing graft formation when mixed with autogenous bone graft, heart bypass surgery, and the treatment of chronic skin and soft-tissue ulcers.[76-78]

The clinical reports are predominantly case studies or limited case series. No published level-1 clinical data supporting the use of PRP is available.

Orthopaedic applications. Although *in vitro* studies have shown a significant relationship between the application of PRP and the proliferation of adult mesenchymal stem cells, the proliferation of fibroblasts and the production of extracellular matrix,[18,57,58,79] its use in trauma and orthopaedic procedures still lacks the support of randomised controlled trials. Further research in basic science and clinical use is needed to define the treatable musculoskeletal conditions, methods of administration and the ideal patient population.

Tendon. Buffered PRP has been used as an alternative to surgery in patients with lateral epicondylitis who had not responded to conservative treatment. In Mishra's series,[80] 15 patients showed significant improvement with a single injection, and this was sustained over time with no reported complications.

In a case-control study, Sánchez et al[81] investigated the effect of PRP in ruptures of the tendo Achillis in athletes who underwent open repair. The procedure was undertaken in conjunction with a preparation rich in growth factors (PRGF) in six athletes and compared retrospectively with a matched group who had the conventional surgical procedure. Those receiving PRGF recovered their range of movement, showed no wound complications and took less time to resume training.[81]

The potential of using PRP in repair of the rotator cuff was evaluated in a pilot study by Randelli et al.[82] After repair of the tear, 14 patients received intra-operative autologous PRP combined with an autologous thrombin component. They were followed up for 24 months and demonstrated a significant reduction in their pain score and significant increases in functional scoring.

Bone. There are few clinical studies examining the role of PRP in bone healing after orthopaedic trauma.[76,83] Most have been related to the use of PRP in oral and cranial surgery. Currently, it is common to combine the platelet-rich material with autograft, allograft, demineralised bone matrix or other graft material to fill bony defects in the mandible or cranium.[84]

Lowery, Kulkarni and Pennisi[85] administered PRP and autogenous bone grafts during spinal fusion with good results, obtaining union in all their patients. Kitoh et al[86] used PRP and bone marrow cells (BMCs) during osteogenesis distraction in three patients, and observed callus formation between days 34 and 47 in all patients. This encouraged them to increase distraction to 1.5 mm per day. Acceleration of bone formation was observed. The authors point out that because they used two osteo-inductive materials, it is very hard to estimate the effect of PRP alone.

In another series, the levels of PDGF and TGF-β were measured in the fracture haematoma of 24 patients who had fresh fractures of the foot and ankle. No evidence for the presence of these proteins was found in seven patients in whom the fractures failed to unite. After application of the PRC to the nonunions during revision operations, radiographic union was observed by an average of 8.5 weeks.[87]

The use of percutaneous injection of autologous platelet-rich gel as a minimally invasive alternative to open grafting in the treatment of delayed and nonunion was explored by Bielecki, Gazdik and Szczepanski.[88] Of their cohort of 32 patients, 12 had delayed union and 20 nonunion. Union was achieved in all cases of delayed union. In the nonunion group, union was observed in 13 of 20 cases and the average time to union was 10.3 weeks after injection with PRP. Healing was only achieved when the average time from the initial operation to injection with PRP was < 11 months.

Joint arthroplasty. Biological materials used to assist in haemostasis after total joint replacement have been the subject of recent research. In a retrospective review of 98 total knee replacements, 61 had PRP applied intra-operatively to exposed tissues, synovium and the lining of the wound at closure. The patients receiving PRP had less post-operative blood loss, fewer oral and intravenous narcotic requirements, a greater range of movement at discharge, and a shorter hospital stay than those who did not have PRP applied to their wound. This study suggests that direct application of PRP to the operative site after knee replacement seals the tissues and delivers platelets directly to the wound.[89]

Another potential application in trauma or total joint arthroplasty involves the use of PRP at the interface between the implant and the bone. With the decline in the use of cement, and the corresponding increased use of press-fit implants, PRP may promote earlier and more complete osteo-integration of implants into host bone. In a randomised controlled trial on 70 patients, Okuda et al[90] demonstrated that a combination of PRP and hydroxyapatite (HA), compared to HA with saline, led to a significantly more favourable clinical improvement in intrabony periodontal defects. They suggested that porous HA lacks the cells and growth factors present in bone graft, but that PRP would potentially diminish this disadvantage. This technique is currently being applied in oral surgery, where implants are placed into extraction sites augmented with PRP.[84]

Diabetic fractures. The association between diabetes mellitus and impaired bony healing has been documented in clinical and experimental settings. Several clinical series have noted that the healing time for diabetic patients is approximately twice as long as that of non-diabetic patients.[91] In addition, diabetic patients undergoing elective arthrodesis have a significantly increased incidence of delayed union, nonunion and pseudarthrosis.[92]

In a diabetic fracture model study, a significant reduction in PDGF, TGF-β1, insulin-like growth factor and VEGF expression was demonstrated in the fracture callus compared to that in non-diabetics.[93,94] The application of PRP restored early cell proliferation during healing to levels comparable to those in non-diabetic controls. The percutaneous injection of PRC normalised early diabetic fracture callus, but the biomechanical properties were only partially restored in the late callus.[95] These observations are consistent with those seen clinically in 50 diabetic patients with a Charcot foot who showed improved healing and fewer complications after ankle fusion when treated with PRP.[96]

Wound healing. As early as in 1990, autologous human platelet-derived wound healing factors (HPDWHF) were proposed to regulate wound healing of recalcitrant skin ulcers by promoting the formation of granulation tissue in the early healing phase.[97] This conclusion was based on a study of 23 patients with 27 skin ulcers who had shown no signs of healing after an average of 25 weeks of conventional wound care. At an average of ten weeks after the application of HPDWHF 100% healing was achieved. In 2001, Margolis et al[97] published a retrospective study analysing the results in 26 599 patients with diabetic neuropathic foot ulcers who had been treated with an autologous platelet releasate. The review suggested that platelet releasate provided with standardised care was more effective than standard care alone.

In another study on 171 patients with 355 wounds, a 78% rate of limb salvage was achieved in those for whom amputation was initially recommended.[98] Fibrin acts as a scaffold for epithelial migration and, along with the growth factors, accelerates the development of granulation tissue and epithelialisation, leading to a less prolonged crusting phase, less pain and an earlier return to normal activity.

Potential risks of PRP. Because PRP is prepared from autologous blood it is inherently safe, and any concerns regarding transmission of diseases such as HIV, hepatitis, or Creutzfeld-Jakob disease, or of immunogenic reactions that exist with preparations of allograft or xenograft, are eliminated.[54] However, the activation of PRP involves using calcium chloride and bovine thrombin preparations, which contain bovine factor V. The systemic use of bovine thrombin in cardiovascular surgery to promote clotting has been reported to be associated with coagulopathies resulting from cross-reactivity of anti-bovine factor V antibodies with human factor V.[99,100] The bovine thrombin preparations used in these cases were of high dose (> 10 000 units) and were applied directly to open wounds, where absorption into the systemic circulation is certain. There have been no similar reports since 1997 owing to the use of highly purified bovine thrombin. The very small dose of bovine thrombin (< 200 units) used to activate PRP before application will be consumed during clot formation and digested by macrophages. Hence, bovine thrombin-activated PRP does not produce anti-factor V antibodies. There are some alternatives to bovine thrombin. Some authors have noted that PRP gel can only be formed with the addition of calcium, but this usually takes longer to complete.[50,52] An effective alternative is thrombin receptor agonist peptide (TRAP), which mimics the effect of thrombin.[101]

Based on the inconclusive results in the literature, this review cannot provide solid evidence in favour of the application of PRP in trauma and orthopaedic surgery. However, because the majority of the clinical trials have shown encouraging outcomes, further controlled clinical trials to elucidate the beneficial effects of PRP are warranted.

This study was supported by the NIHR Biomedical Research Centre, Oxford.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

1. **Man D, Plosket H, Winland-Brown JE.** The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg* 2001;107:229-37.
2. **Matras H.** Effect of various fibrin preparations on reimplantations in the rat skin. *Osterr Z Stomatol* 1970;67:338-59 (in German).
3. **Gibble J, Ness P.** Fibrin glue: the perfect operative sealant? *Transfusion* 1990;30:741-7.
4. **Sanchez AR, Sheridan PJ, Kupp LI.** Is platelet-rich plasma the perfect enhancement factor?: a current review. *Int J Oral Maxillofac Implants* 2003;18:93-103.
5. **Whitman DH, Berry RL, Green DM.** Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg* 1997;55:1294-9.
6. **Einhorn TA.** The science of fracture healing. *J Orthop Trauma* 2005;19(Suppl):4-6.
7. **No authors listed.** EMBASE. http://www.EMBASE.com (date last accessed 30 June 2009).
8. **No authors listed**. CINAHL. http://ebscohost.com/cinahl (date last accessed 30 June 2009).
9. **No authors listed.** MEDLINE. http://ncbi.nlm.nih.gov/pubmed (date last accessed 30 June 2009).
10. **Marx RE, Carlson ER, Eichstaedt RM, et al.** Platelet rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638-46.

11. **Weibrich G, Kleis WKG, Hafner G, Hitzler WE.** Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. *J Cranio Maxillofacial Surg* 2002;30:97-102.
12. **Smrke D, Gubina B, Damanovic D, Rozman P.** Allogeneic platelet gel with autologous cancellous bone graft for the treatment of a large bone defect. *Eur Surg Res* 2007;39:170-4.
13. **Harrison P, Cramer EM.** Platelet alpha-granules. *Blood Rev* 1993;7:52-62.
14. **Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT.** Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004;91:4-15.
15. **Bucholz RW, Einhorn TA, Marsh JL.** Bone and joint healing. In: Bucholz RW, Heckman JD, Court-Brown C, eds. *Rockwood & Green's fractures in adults*. Sixth ed. Lippincott Williams & Wilkins, 2006:300-11.
16. **Marx RE.** Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent* 2001;10:225-8.
17. **Marx RE.** Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 2004;62:498-93.
18. **Schliephake H.** Bone growth factors in maxillofacial skeletal reconstruction. *Int J Oral Maxillofac Surg* 2002;31:469-84.
19. **van den Dolder J, Mooren R, Vloon AP, Stoelinga PJ, Jansen JA.** Platelet-rich plasma: quantification of growth factor levels and the effect on growth and differentiation of rat bone marrow cells. *Tissue Eng* 2006;12:3067-73.
20. **Eppley BL, Woodel JE, Higgins J.** Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004;114:1502-8.
21. **Polasek J, Richardson M, Moore MA, Blajchman MJ.** Evidence for an alternative mechanism of platelet secretion involving peripheralization of secretory granules and formation of membrane associated multivesicular structures. *Thrombosis Research* 1987;45:771-82.
22. **Antoniades HN.** Human platelet derived growth factor (PDGF): purification of PDGF-I and PDGF-II and separation of their reduced subunits. *Proc Nat Acad Sci USA* 1981;78:7314-17.
23. **Rappolee DA, Mark D, Banda MJ, Werb Z.** Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping. *Science* 1988;241:708-12.
24. **Sitaras NM, Sariban E, Pantazis P, Zetter B, Antoniades HN.** Human iliac artery endothelial cells express both genes encoding the chains of platelet-derived growth factor (PDGF) and synthesize PDGF-like mitogen. *Cell Physiol* 1987;132:376-80.
25. **Hauschka PW, Mavrakos AE, Iafrati MD, Doleman SE, Klagsburn M.** Growth factors in bone matrix. *J Biol Chem* 1986;261:12665-74.
26. **Ross R, Raines EW, Bowen-Pope DF.** The biology of platelet-derived growth factor. *Cell* 1986;46:155-69.
27. **Raines EW, Ross R.** Platelet-derived growth factor. I: high yield purification and evidence for multiple forms. *J Biol Chem* 1982;257:5154-60.
28. **Oates TW, Rouse CA, Cochran DL.** Mitogenic effects of growth factors on human periodontal ligament cells in vitro. *J Periodontal* 1993;64:142-8.
29. **Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ.** Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors in vitro. *J Periodontal* 1992;63:515-25.
30. **Yang D, Cheng J, Jing Z, Jin D.** Platelet-derived growth factor (PDGF)-AA: a self-imposed cytokine in the proliferation of human fetal osteoblasts. *Cytokine* 2000;12:1271-4.
31. **Celeste AJ, Iannazzi JA, Taylor RC, et al.** Identification of transforming growth factor-beta to family members present in bone inductive protein purified bovine bone. *Proc Natl Acad Sci USA* 1990;87:9843-7.
32. **Duke PT, Hansen P, Iwata KK, Pieler C, Foulkes JG.** Identification of another member of the transforming growth factor type b gene family. *Proc Natl Acad Sci USA* 1988;85:4715-19.
33. **Wrana JL, Macho M, Hawrylyshyn B, et al.** Differential effects of transforming growth factor-beta on the synthesis of extracellular matrix proteins by normal fetal rat calvarial bone cell populations. *J Cell Biol* 1988;106:915-24.
34. **Bonewald LF, Mundy GR.** Role of transforming growth factor-beta in bone remodeling. *Clin Orthop* 1990;250:261-76.
35. **Mohan S, Baylink DJ.** Bone growth factors. *Clin Orthop* 1991;263:30-48.
36. **Hock JM, Gentrella M, Canalis E.** Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. *Endocrinology* 1988;122:254-60.
37. **McCarthy T, Centrella M, Canalis E.** Further biochemical and molecular characterization of primary rat parietal bone cell cultures. *J Bone Miner Res* 1988;3:401-8.
38. **Joseph BK, Savage NW, Daley TJ, Young WG.** In situ hybridization evidence for a paracrine/autocrine role for insulin-like gtowth factor-I in tooth development. *Growth Factors* 1996;13:11-17.
39. **Cohen S.** Isolation of submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. *J Biol Chem* 1962;237:1555-62.
40. **Hom DB, Maisel RH.** Angiogenic growth factors: their effects and potential in soft tissue wound healing. *Ann Otol Rhinol Laryngol* 1992;101:349-54.
41. **Maurer AM, Zhou B, Han ZC.** Roles of platelet factor 4 in hematopoiesis and angiogenesis. *Growth Factors* 2006;24:242-52.
42. **Ferrara N, Gerber HP.** The role of vascular endothelial growth factor in angiogenesis. *Acta Haematol* 2001;106:148-56.
43. **Herbst RS.** Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Bio Phys* 2004;59(Suppl 2):21-6.
44. **Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE.** Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. *Bone* 2004;34:665-71.
45. **Choi BH, Zhu SJ, Kim BY, et al.** Effect of platelet rich plasma (PRP) concentration on the viability and proliferation of alveolar bone cells: an in vitro study. *Int J Oral Maxillofac Surg* 2005;34:420-4.
46. **Marx RE.** Platelet concentrate: a strategy for accelerating and improving bone regeneration. In: Davies JE, ed. *Bone engineering*. University of Toronto, 2000:447-53.
47. **Weibrich G, Kleis WK.** Curasan PRP kit vs PCCS PRP system: collection efficiency and platelets counts of two different methods for the preparation of platelet rich plasma. *Clin Oral Implants* 2002;13:437-43.
48. **Kevy SV, Jacobson MS.** Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corp Technol* 2004;36:28-35.
49. **Marlovits S, Mousavi M, Gabler C, Erdös J, Vécsei V.** A new simplified technique for producing platelet-rich plasma: a short technical note. *Eur Spine J* 2004;13(Suppl 1):102-6.
50. **Landesberg R, Roy M, Glickman RS.** Quantification of growth factors levels using a simplified method of platelet rich plasma gel preparation. *J Oral Maxillofac Surg* 2000;58:297-300.
51. **Schoendorfer DW, Williamson LH, Sheckler VL, Fitzgerald BP.** Platelet collection with the autopheresis-C apheresis system. *Vix Sang* 1990;58:100-5.
52. **Everts P, Mahoney C, Hoffmann J, et al.** Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors* 2006;24:165-71.
53. **Pietrzak WS, Eppley BL.** Platelet rich plasma: biology and new technology. *J Craniofac Surg* 2005;16:1043-54.
54. **Man D, Plosker H, Winland-Brown JE.** The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Recon Surg* 2001;107:229-37.
55. **Castelnovo L, Dosquet C, Gaudric A, Sahel J, Hicks D.** Human platelet suspension stimulates porcine retinal glial proliferation and migration in vitro. *Invest Opthalmol Vis Sci* 2000;41:601-9.
56. **Soffer E, Ouhayoun JP, Dosquet C, Meunier A, Anagnostou F.** Effects of platelet lysates on select bone cell functions. *Clin Oral Implants Res* 2004;15:581-8.
57. **Lucarelli E, Fini M, Beccheroni A, et al.** Stromal stem cells and platelet-rich plasma improve bone allograft integration. *Clin Orthop* 2005;435:62-8.
58. **Slater M, Patava J, Kingham K, Mason RS.** Involvement of platelets in stimulating osteogenic activity. *J Orthop Res* 1995;13:655-63,
59. **Oprea WE, Karp JM, Hosseini MM, Davies JE.** Effect of platelet releasate on bone cell migration and recruitment in vitro. *J Craniofac Surg* 2003;14:292-300.
60. **Klein MB, Yalamanchi N, Pham H, et al.** Flexor tendon healing in vitro: effects of TGF-beta on tendon cell collagen production. *J Hand Surg [Am]* 2002;27:615-20.
61. **Schnabel LV, Mohammed HO, Miller BJ, et al.** Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res* 2007;25:230-40.
62. **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.
63. **de Mos M, van der Windt AE, Jahr H, et al.** Can platelet-rich plasma enhance tendon repair?: a cell culture study. *Am J Sports Med* 2008;36:1171-8.
64. **Kim ES, Park EJ, Choung PH.** Platelet concentration and its effect on bone formation in calvarial defects: an experimental study in rabbits. *J Prosthet Dent* 2001;86:428-33.
65. **Fennis JP, Stoelinga PJ, Jansen JA.** Mandibular reconstruction: a histological and histomorphometric study on the use of autogenous scaffolds, particulate corticocancellous bone grafts and platelet rich plasma in goats. *Int J Oral Maxillofac Surg* 2004;33:48-55.
66. **Tomoyasu A, Higashio K, Kanomata K, et al.** Platelet-rich plasma stimulates osteoblastic differentiation in the presence of BMPs. *Biochem Biophys Res Commun* 2007;361:62-7.
67. **Torres J, Tresguerres I, Tamimi F, et al.** Influence of platelet-rich plasma on bone regeneration: a histomorphometric study in rabbit calvaria. *Int J Oral Maxillofac Implants* 2007;22:563-8.
68. **Aspenberg P, Virchenko O.** Platelet concentrate injection improves Achilles tendon repair in rats. *Acta Orthop Scand* 2004;75:93-9.

69. 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.

70. Kovacevic D, Rodeo SA. Biological augmentation of rotator cuff tendon repair. *Clin Orthop* 2008;466:622-33.

71. Aghaloo TL, Moy PK, Freymiller EG. Investigation of platelet-rich plasma in rabbit cranial defects: a pilot study. *J Oral Maxillofac Surg* 2002;60:1176-81.

72. Chaput CD, Patel KV, Brindley GW, et al. Influence of a platelet concentrate on prosthetic bone ingrowth in a rabbit model. *J Surg Orthop Adv* 2007;16:159-63.

73. Ranly DM, McMillan J, Keller T, et al. Platelet-derived growth factor inhibits demineralzed bone matrix-induced intramuscular cartilage and bone formation: a study of immunocompromised mice. *J Bone Joint Surg [Am]* 2005;87-A:2052-64.

74. Ranly DM, Lohmann CH, Andreacchio D, Boyan BD, Schwartz Z. Platelet-rich plasma inhibits demineralised bone matrix-induced bone formation in nude mice. *J Bone Joint Surg [Am]* 2007;89-A:139-47.

75. Tayapongsak P, O'Brien DA, Monteiro CB, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. *J Oral Maxillofac Surg* 1994;52:161-5.

76. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: a review of biology and applications in plastic surgery. *Plast Reconstr Surg* 2006;118:147-59.

77. Knighton DR, Phillips GD, Fiegel VD. Wound healing angiogenesis: indirect stimulation by basic fibroblast growth factor. *J Trauma* 1990;30(Suppl 12):134-44.

78. DelRossi AJ, Cernaianu AC, Vertrees RA, et al. Platelet-rich plasma reduces postoperative blood loss after cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1990;100:281-6.

79. Liu Y, Kalen A, Risto O, et al. Fibroblast proliferation due to exposure to a platelet concentrate in vitro is pH dependent. *Wound Repair Regen* 2002;10:336-40.

80. Mishra A, Pavelko T. Treatment of chronic elbow tendinosus with buffered platelet-rich plasma. *Am J Sports Med* 2006;34:1774-8.

81. Sánchez M, Anitua E, Azofra J, et al. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med* 2007;35:245-51.

82. Randelli PS, Arrigoni P, Cabitza P, Volpi P, Maffulli N. Autologous platelet rich plasma for arthroscopic rotator cuff repair: a pilot study. *Disabil Rehabil* 2008:1-6.

83. Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstructiion: a histological and histomorphometric study on the use of autogenous scaffolds, particulate corticocancellous bone grafts and platelet rich plasma in goats. *Int J Oral Maxillofac Surg* 2004;33:48-55.

84. Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638-46.

85. Lowery GL, Kulkarni S, Pennisi AE. Use of autologous growth factors in lumbar spinal fusion. *Bone* 1999;25(Suppl 2):47-50.

86. Kitoh H, Kitakoji T, Tsuchiya H, Katoh M, Ishiguro N. Distraction osteogenesis of the lower extremity in patients with achondroplasia/hypochondroplasia treated with transplantation of culture-expanded bone marrow cells and platelet-rich plasma. *J Pediatr Orthop* 2007;27:629-34.

87. Gandhi A, Bibbo C, Pinzur M, Lin S. The role of platelet-rich plasma in foot and ankle surgery. *Foot Ankle Clin* 2005;10:621-37.

88. Bielecki T, Gazdzik TS, Szczepanski T. Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. *Eur Surg Res* 2008;40:289-96.

89. Gardner MJ, Demetrakopoulos D, Klepchick PR, Mooar PA. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty: an analysis of the haemoglobin, narcotic requirement and range of motion. *Int Orthop* 2007;31:309-13.

90. Okuda K, Tai H, Tanabe K, et al. Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: a comparative controlled clinical study. *J Periodontol* 2005;76:890-8.

91. Loder RT. The influence of diabetes mellitus on the healing of closed fractures. *Clin Orthop* 1988;232:210-16.

92. Stuart MJ, Morrey BF. Arthrodesis of the diabetic neuropathic ankle joint. *Clin Orthop* 1990;253:209-11.

93. Gandhi A, Doumas C, O'Connor JP, Parsons JR, Lin SS. The effects of local platelet rich plasma delivery on diabetic fracture healing. *Bone* 2006;38:540-6.

94. Tyndall WA, Beam HA, Zarro C, O'Connor JP, Lin SS. Decreased platelet derived growth factor expression during fracture healing in diabetic animals. *Clin Orthop* 2003;408:319-30.

95. Grant WP, Jerlin EA, Pietrzak WS, Tam HS. The utilization of autologous growth factors for the facilitation of fusion in complex neuropathic fractures in the diabetic population. *Clin Podiatr Med Surg* 2005;22:561-84.

96. Herouy Y, Mellios P, Bandemir E, et al. Autologous plateler-derived wound healing factor promotes angiogenesis via $\alpha v\beta 3$ integrin expression in chronic wounds. *Int J Mol Med* 2000;6:515-19.

97. Margolis DJ, Kantor J, Santanna J, et al. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 2001;24:483-8.

98. Knighton DR, Fiegel VD, Austin LL, Gresi KF, Butler EL. Classification and treatment of chronic nonhealing wounds: successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann Surg* 1986;204:322-9.

99. Landesberg R, Moses M, Karpatkin M. Risks of using platelet rich plasma gel. *J Oral Maxillofac Surg* 1998;56:1116-17.

100. Cmolik BL, Spero JA, Magovern GJ, Clark RE. Redo cardiac surgery: late bleeding complications from topical thrombin induced factor five deficiency. *J Thorac Cardiovasc Surg* 1993;105:222-7.

101. Landesberg R, Burke A, Pinsky D, et al. Activation of platelet-rich plasma using thrombin receptor agonist peptide. *J Oral Maxillofac Surg* 2005;63:529-35.