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GROWTH FACTOR CONTENT IN PRP AND THEIR APPLICABILITY IN MEDICINE

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This paper reviews available reports on the advantages and possibilities of clinical use of platelet-rich plasma preparations, with particular emphasis on platelet growth factors.

Platelets, an important reservoir of growth factors in the body, play an important role in many processes such as coagulation, immune response, angiogenesis and the healing of damaged tissues. Numerous proteins are contained in the α-granules of platelets: platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet factor interleukin (IL), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor IGF and fibronectin.

The development of methods and systems for blood and cell sorting (e.g. CAPSS - compact advanced platelet sequestration system Elektromedics 500, PCCS - platelet concentrate collection system Curasan) have made it possible to obtain significant concentrations of platelets (even by 338%) and high concentrations of growth factors, in a form of sterile mass that can be used immediately for clinical purposes. Platelet-rich plasma (PRP; autologous platelet-rich plasma - APRP) are platelet concentrates made of autogenous blood with a high number of platelets in a small volume of plasma. The clinical efficacy of platelet concentrates depends mainly on the number of platelets and the concentration of their growth factors, which act as transmitters in most processes in tissues, particularly in healing where they are responsible for proliferation, differentiation, chemotaxis and tissue morphogenesis. They operate as part of autocrine, paracrine and endocrine mechanisms.

Growth factors derived from centrifuged blood were first used in patients with chronic skin ulcers. The clinical use of PRP for a wide variety of applications has been reported mostly in oral and maxillo-facial surgery, orthopedic surgery, treatment of soft tissue diseases and injuries, treatment of burns, hard-to-heal wounds, tissue engineering and implantology.

Platelet Concentrate Products

For over two decades we have seen a growing interest in blood as a source of substances that could potentially accelerate the healing of tissues and wounds, and the possible use of blood-based products in the complex interactions between cells of connective tissue, epithelial cells, immune cells and blood morphotic elements that accompany healing and regeneration. Current treatment methods use the advantages of tissue engineering (biomimetics) that allow emulation of embryogenetic processes in tissue healing. Proper tissue regeneration requires three components that are completely dependent on one another: media, cells and extracellular matrix components that include growth factors, morphogens, adhesins, hormones and vitamins (1). The process of tissue healing is divided into three phases: inflammation,

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proliferation and remodeling. Blood platelets play a key role in the phase of inflammation: they are responsible for hemostasis,the release of signal particles that affect the developing inflammatory processes, as well as the growth, differentiation and migration of cells taking part in tissue reconstruction. Platelets, the smallest blood cells, with a diameter of 2-4 μm, devoid of a nucleus, discoid-shaped and with megakaryocytes as their stem cells, serve a variety of critical functions including hemostasis (coagulation), inflammation, antimicrobial host defense, angiogenesis, and wound healing, and are subject to daily consumption that reaches 25% inder conditions of physiological equilibrium. Platelet activity is related to their age; young platelets exhibit glycolytic activity and the capacity for biosynthesis of glycogen and proteins, mainly thrombosthenin and glycoprotein.

Once a platelet is exhausted and dies off, the macrophages which arrived in the region via vascular in-growth stimulated by the platelets, assume the function of wound healing regulation by secreting some of the same growth factors (2).

Recent years have seen a growing demand for platelets and their concentrates, associated with the increasing number of cardiac operations, organ transplants and cancer treatment procedures associated with their use.

Platelets are a potential source of therapeutically active proteins such as mitogenic, chemotactic, adhesive, angiogenic and antiangiogenic proteins, and neurothrophic factors. Recently developed procedures can be used to create platelet-rich plasma (PRP), while many techniques are available for collecting platelet concentrates, although each method may lead to a different product with different biology and potential uses. The term PRP firstly relates to platelet concentrates used in transfusion medicine, but nowadays it has started to be used for all platelet concentrates, regardless of the exact composition of the products, resulting in a certain confusion in terms of terminology. There is a lack of clear and uniformly accepted terminology, making a source of confusion in the field. Knowledge and terminology regarding the preparation, differentiation and use of blood products have been greatly enriched and structured thanks to the contributions of the teams under Marx, Anitua and Dohaan Ehrenfest (2-6).

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. During the preparation procedure, centrifuging at varying speeds accelerates the sedimentation of heavier cells such as white blood cells and red blood cells, while platelets (which sediment at a lower rate) remain floating within the plasma fraction at the top of the tubes. Usually 2 spins are used. The first spin (hard spin) separates the platelet poor plasma (PPP) from the red fraction and platelet rich plasma (PRP). The second spin (soft spin) separates the red fraction from the PRP (7).

PRP is prepared in a laboratory, surgical or dental suite from blood collected in the immediate preoperative period. Heterologous or allogenic materials carry the potential risks of infection, immune responses and pathogen transmission and are not appropriate for clinical feasibility. At the time of application, the PRP is combined with an equal volume of sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate) and 100 U/mL of sterile bovine thrombin (an activator that allows polymerization of the fibrin into an insoluble gel, causing the platelets to degranulate and release the indicated mediators and cytokines) (8).

Very often the term PRP is preferred to autologous platelet gel, plasma-rich growth factors (PRGFs), or autologous platelet concentrate. Pure platelet-rich plasma (P-PRP) and leukocyte- and platelet- rich plasma (L-PRP) are liquid platelet suspensions either with or without leukocytes. PRP gel and L-PRP gel are formed by mixing PRP or L-PRP, derived from centrifuging autologous whole blood, with thrombin, calcium chloride, batroxobin or other activators, which then start to polymerize into a fibrin gel presenting a light and fragile architecture, to be used in various applications with apparent clinical success (9).

PRGF (plasma, rich in growth factors) has a moderated platelet concentration and does not contain leukocytes, with the aim of avoiding the proinflammatory effects of the proteases and acid hydrolases contained in the white blood cells (4).

Pure Platelet-Rich Fibrin (P-PRF) and Leukocyte- and Platelet-Rich Fibrin (LPRF) are solid fibrin biomaterials without or with leukocytes, respectively. They only exist in an activated form, the activation being part of their production process, and present a stronger and more stable fibrin polymerization than the PRP gel families.

P-PRP and L-PRP without activation can be injected as liquid preparations in regenerative medicine treatment, mostly in nonoperative sports medicine. The platelets slowly release their contents during several days to relieve pain and stimulate regeneration of damaged tissues. P-PRP gel, L-PRP gel, P-PRF, and L-PRF are used like fibrin-based solid biomaterials for their hemostatic and adhesive properties. The strong matrix architecture of P-PRF and L-PRF allows for the release of key growth factors and matrix molecules over several weeks in some conditions (5, 10, 11).

Although the definition of platelet rich plasma, proposed by Marx in 2001, mentions 1 million platelets in one microlitre, measured in the standard 6-mL aliquot, a 4-5 fold increase in the concentration of platelets

compared to whole blood (6), in clinical practice the number of platelets in the preparations may even be 14 times greater than in the whole blood (13).

PRP containing approximately 1 million platelets/ μL is recommended as advisable according to in vivo experiments in bone regeneration that showed that an advantageous biological effect was observed with the application of PRP at this platelet concentration (14), while higher concentrations could actually be an inhibitory factor. Additionally Graziani et al. suggest that fibroblasts can be optimally stimulated to increase proliferation at lower platelet concentrations (15). On the contrary, Lui et al. (16) showed that fibroblast proliferation and type I collagen production were also enhanced by increasing platelet concentrations, and that much of the response was pH dependent with the best responses occurring at more acidic pH levels.

Many factors can lead to platelet activation during the preparation of platelet concentrates. Platelets may undergo activation during collection, processing and storage of platelet concentrates (17). The latest study suggests that the concentration of grow factors of platelet concentrates can be retained for at least 6 months in storage at -70°C, and GF of platelet concentrates can be obtained by means of direct freezing, regardless of whether thrombin activation is used. Freeze-thaw procedures are a common method for releasing intracellular GF (18). The use of EDTA anticoagulated samples is potentially more harmful than citrate in the preparation of PRP gel. Although EDTA gave greater yields of platelets. they appeared damaged by the presence of EDTA (19).

Despite many experimentally confirmed advantages resulting from PRP use, it can be noticed that due to the different conditions in each experiment, variable results are unavoidable. This review describes the scientific and mechanistic bases of the biological activities and clinical possibilities of using PRP technology. A literature search was conducted using the MEDLINE/PubMed database for appropriate articles in English language. Databases were searched from 1985 up to 2011. The articles were successively searched by keyword or title using the following keywords: "PRP", "platelet derived growth factor", "platelet-rich plasma", "autologous gel", "platelet gel", and "growth factors".

Growth factors in PRP

Platelet α-granules have been shown to contain mitogenic and chemotactic growth factors (GF) and associated healing molecules in an inactive form, important in wound healing, such as platelet-derived growth factor (PDGF), transforming growth factors β1, β2, β3 (TGF-β1, TGF-β2, TGF-β3), platelet-derived angiogenesis factor (PDAF), insulin-like growth factor 1 (IGF-1), platelet factor 4 (PF-4), epidermal growth factor (EGF), epithelial cell grow factor (ECGF), vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF) and others cytokines. Additionally, plasma fluid also contains a number of biologically active proteins such as growth factor IGF-I and hepatocyte growth factor (HGF). During normal wound healing, trapped platelets become activated and degranulate, resulting in a release of the α-granule content. In this process, the granules fuse to the platelet cell membrane where the protein growth factor is moved to a bioactive state by the addition of histones and carbohydrate side chains to these proteins. The secreted growth factors immediately bind to the external surface of cell membranes in the graft, flap, or wound via transmembrane receptors in mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells, and epidermal cells (2,6).

They mediate many of the cellular functions including cell migration, proliferation, differentiation, metabolism and apoptosis, as well as cellular cycle (2, 20, 21). Platelets are therefore expected to stimulate damaged tissues and to regulate local inflammatory processes. The effects of these growth factors on cell behavior and on wound healing have been thoroughly studied. Platelet counts and PRP growth factor content are likely to depend on the particular technique used in obtaining the PRP. Platelet activation during preparation of the platelet concentrate can result in early α-granule release and loss of the growth factors during the collection process. It is therefore critical to recognize that each platelet-rich plasma preparation method may differ in regard to platelet number, platelet activation rate and growth factor profile.

Theoretical levels of platelet-derived growth factors in PRP might be expected to depend on the number of platelets involved, but the study by Weibrich et al. did not show a statistically significant correlation between PRP platelet count and growth factor level (22), and important variations in GF concentrations were detected between individuals having very similar platelet counts (23, 24). Martineau et al. observed inter-individual variations in GF concentration, especially evident for VEGF (up to a 900-fold difference between donors), and also did not find a correlation between concentrations of different GF within the same donor (24).

Literature data demonstrates that platelet counts in either whole blood or PRP are not predictors for the resulting growth factor levels in PRP. Scientists concluded that this result was connected with high individual variability in cellular production or storage of cytokines. Several parameters influence the relationship between platelet concentration and growth factor levels, such as sufficient activation of platelets to initiate α-granule release, white blood cell count, plasma growth factor

contamination, and collection of growth factors from the fibrin clot. Fréchette et al. recently demonstrated that the release of GF is significantly regulated by the amount of calcium and thrombin added to the PRPs. The addition of calcium and thrombin to PRPs had different effects on extracellular GF concentration. EGF and Ang-2 levels increased immediately after treatment, IGF-1 decreased slowly, and a delayed increase in IL-1 concentration was observed over time. These patterns were observed for all donors, suggesting that calcium and thrombin regulate GF release, synthesis, and/or degradation in stereotyped patterns that are specific to each growth factor (25).

It has been shown that PDGF, TGFβ1, VEGF and EGF cytokines were all significantly greater in platelet-rich plasma samples than in the whole blood baseline samples. On average, the PDGF-BB isoform increased from 3.3 ± 0.9 ng/ml to 17 ± 8 ng/ml, TGF-β1 increased from 35 ± 8 ng/ml to 120 ± 42 ng/ml, VEGF increased from 155 ± 110 pg/ml to 955 ± 1030 pg/ml, and EGF increased from 129 ± 61 pg/ml to 470 ± 317 pg/ml. Weibrich et al. (22, 26), in their examination of the concentrations of individual grow factors in PRP produced by discontinuous flow separation, showed the presence of three growth factors: PDGF-AB (117.5763.4 ng/ml), TGF-b1 (169.47 84.5 ng/ml) and IGF-I (84.2723.6 ng/ml). PDGFBB (9.977.5 ng/ml) and TGF-b2 (0.470.26 ng/ml) were present only in small amounts. The discontinuous cell separation method for PRP production yielded an approximately 5-fold increase in platelet concentration when compared with the initial platelet concentration in the donor blood. Pearson's correlation coefficient between platelets in PRP and in whole blood in the mentioned study was significant (r=0.77), however the Pearson's correlation coefficients for whole blood platelets, PRP platelets, and growth factor contents demonstrated little relationship between these parameters (r ≤0.35). In addition, they showed a slight decrease of IGF-I in PRP with age.

Fréchette et al. (25) demonstrated that the low level of EGF detected in supernatants from nonactivated PRPs (57 ± 77 pg/mL) prepared with the Platelet Concentrate Collection System (PCCS) increased in response to addition of calcium and thrombin (4.7- to 11-fold; $p < 0.005$) even up to 595 ±195 pg/mL. On the contrary, IGF-1 concentrations in supernatants from non-activated PRPs were significantly higher compared with all other GF and did not increase after the addition of calcium and thrombin (5.5 ± 2.1 ng/Ml). Mean TGFα concentrations were either not detectable or were very close to the detection threshold. In the study conducted by Martineau et al. ELISA assays revealed that bFGF and VEGF were present at low concentrations in platelet concentrate (PC) supernatants prepared using the PC Collection System. bFGF concentrations ranging from 6 to 46 pg/ml in non-activated PC, and that treatment with high calcium and thrombin concentrations contributed to significant and rapidly increases in bFGF levels, being very similar for all of the donors (mean increases: calcium:325%, thrombin:745%), with maximum concentrations detected 1h after activation. Treating PC with low calcium and thrombin concentrations tended to reduce the total amount of bFGF. VEGF were detected in supernatants from non-activated PC at 57.2 pg/ml, while calcium and thrombin concentrations induced a moderate increase (57%) regardless of their concentrations. The amounts of PDGF-BB ranged from 1.7 to 10.6 ng/ml and were evaluated in non-activated PC in the mentioned study with an increase (range 16–50 ng/ml) following calcium and thrombin incubation. Baseline levels of TGF-b1 were within 0.3–5.3 ng/ml, an 84% increase in TGF-b1 concentration was observed in supernatants from PC treated with calcium and thrombin concentrations (24).

Banks et al. (1998) reported similar VEGF concentrations in thrombin-activated PC (range: 38–505 pg/ml) and in calcium-activated PC (range: 28–369 pg/ml) (27).

The potential loss of bioactivity of the growth factors may be concerned with the storage of PRP, and less than 48h of storage would be allowable for the better retention of platelet properties (28, 29).

One should also remember that plasma as a PRP component is a rich source of many proteins and ions of organic and inorganic particles, and that plasma proteins are also known to be critical components in the healing mechanism of connective tissues.

PRP also contains three proteins in blood known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue and epithelial migration. These cell adhesion molecules are fibrin itself, fibronectin, and vitronectin (7). When plasma is exposed to thrombin, either by the addition of exogenous thrombin or by coming in contact with tissue thromboplastin (also known as tissue factor), the clotting cascade is initiated and platelets are activated resulting in formation of a fibrin clot providing a provisional scaffold for cell migration as well as a reservoir of growth factors (30). However, research by Landesberg et al. showed that the amounts of TGFβ and PDGF present in the platelet-free plasma were minimal, indicating that almost all of the growth factor/cytokine present in the PRP gel is derived from the platelets (19). Additionally, Peterson et al. documented that the concentrations of VEGF, PF-4, PDGF, TSP-1, and bFGF in platelets of normal human subjects differ significantly from those in plasma. The concentrations of the selected angiogenesis regulators investigated in this study were higher in platelets than in plasma (31).

Clinical and experimental significance of platelet

growth factors

Platelet-rich plasma concentrate is applied in many fields of medicine. The general concept of useful PRP is to concentrate blood platelets through centrifugation of whole blood and to apply them with or without direct activation where their healing potential is needed (3). PRP works via degranulation of the granules in platelets which contain the synthesized and prepackaged growth factors. More than 95% of the presynthesized growth factors are secreted within 1 hour, and degranulation in platelets and secretion of growth factors occurs up to 3-5 days after the combination of the platelet-rich plasma with thrombin and calcium ions; the growth factors remain active for 7-10 days. The PRP growth factors never enter the cell or its nucleus, they are not mutagenic, and have no ability to induce tumor formation (6, 12, 32). Platelets in PRP also play a role in host defense mechanisms at the wound site by producing signaling proteins that attract macrophages (33).

Platelet concentrates containing growth factors are applied in clinical practice in various ways. A lot of studies have been performed to investigate the effect of PRP upon tissue regeneration, however, the results are controversial, often due to differing methods of platelet concentrate preparation. Biology and clinical potential of preparations are dependent on their composition and the way the growth factors, matrix and cells are assembled together.

The first attempts to apply the platelet gel in the treatment of bone defects were made by maxillofacial surgeons, demonstrating higher bone density in histomorphometric studies in patients with bone defects filled with bone marrow mixed with PRP gel, compared to patients who used only bone marrow; a significantly greater percentage of trabecular bone was achieved after the addition of PRP (74% with PRP compared with 55% without PRP) (6). Research by Cieślik-Bielicka et al. on the use of PRP in cysts in the mandible confirmed its osteoinductive properties (34). Using autogenous platelet-rich plasma (PRP) is currently being widely studied in applications for dental implants as a method of accelerating the maturation of bone in the mandible (35). Anuita et al. after putting PRP in the alveolus after extraction of teeth, observed a faster bone regeneration and healing of damaged tissue during surgery on oral mucosa (4), and the clinical advantages of PRP use have been highlighted by Marx (2). Kassolis et al. used PRP with freeze-dried bone allografts in sinus floor elevation and alveolar ridge augmentation with good postoperative results (36). These procedures are applicable for improving the contour of the alveolar ridge in relation to ideal pontic papilla esthetics for fixed partial prosthesis, healthy dentoalveolar complex for periodontal attachment and bone for dental implant placement (37). Growth factors exert regulatory effects on the homeostasis of the periodontal tissues and they also have the ability of modifying the response of periodontal soft and hard tissues during the healing processes after their exogenous application (38). In the field of implant dentistry, the application of PRP in addition to autogenous grafts used for these procedures like sinus lifts, ridge augmentations, etc. will promote and accelerate the osseo-integration process. It seems specially benefitted in maxilla, in cases of previous implant failures in type 4 bone in osteoporotic women (7, 12).

PRP has found clinical applications in fully autogenous bone grafts and composites of autogenous bone grafts with a variety of bone substitutes with as little as 20% autogenous bone (39), has shown improved results in sinus lift augmentation grafting (36, 40, 41), in horizontal and vertical ridge augmentations (39), and in ridge preservation grafting (35).

Franchini et al. used a platelet concentrate in fractures, in pseudoarthrosis, bone reconstructions in hip joint arthroplasty, in fibrous dysplasia and osteomyelitis (13). These preparations are also used with good effect in patients from risk groups that undergo ankle arthrodesis (42) and in reconstructive knee surgeries (43, 44).

In continuing inflammation, or directly after operation and application of PRP, the activation of PDGF, VEGF, TGF-β1 and TGF-β2 result in the improved control of cellular metabolism by reducing the formation of oxygen radicals (45).

PRP is used to treat injuries and chronic tendon and ligament pathologies. Factors released from platelets significantly increased the proliferation of tendon cells and also stimulated them to secrete VEGF and HGF to promote angiogenesis and prevent the formation of scar tissue around the tendon. These processes are closely linked with the tendon's ability to heal (46).

The effectiveness of PRP has been reported both after the surgical repair of ruptured tendons and in various tendinopathies (47, 48).

Hiramatsu et al. (2002) examined the effects of reinfusion of autologous platelet concentrate after open heart surgery in patients with noncyanotic congenital heart disease. Reinfusion of freshly prepared autologous PRP was followed by good aggregation responses and low blood loss. The results of that study suggest that this procedure might be useful in open heart surgery to avoid blood transfusions and minimize the need for homologous blood products (49).

Hammond et al. (2009) in an experimental animal study proved that the use of platelet-rich plasma (PRP) concentrate accelerates the healing of muscle tissue, PRP was administered into the injury site in the tibialis anterior muscle (50).

Table 1. *Human and animal clinical applications using different kind of rich platelets preparation the results of the study.*

Study	Investigated human/animal group	Treatment	Results
Kashima et al. (2000)	8 patients underwent nine Thoracic Aortic Aneurysm surgery	PRP transfusion during operations (PRP gained during operation)	Autologous platelet-rich plasma transfusion was an effective way to reduce homologous blood transfusions in thoracic aortic aneurysm surgery.
Man et al. (2201)	20 patients undergoing cosmetic surgery involving the creation of a surgical flap. The types of surgical procedures: face lifts, breast augmentations, breast reductions, and neck lifts.	For each patient the type of surgical procedure and the average area was different. Only of the capillary bed under the flap in each case was the same/autologous fibrin glue=PPP (Platelet-poor plasma), PRP-gel/ 7,5 cc of PPP and PRP	Autologous fibrin glue and platelet gel in cosmetic surgical procedures, resulted in many advantages included: shorter operating times, the elimination of the need for drains, a reduction in the need for compressive dressings, a reduction in pain and postoperative swelling, and improved wound healing with a shorter recovery time.
Froum et al. (2002)	3 patients with bilateral sinus augmentation procedures prior to the placement of the implants.	Experimental side (PRP) and the control side (non-PRP). All six sinuses in this pilot study were grafted with an organic bovine bone (0.25 to 1 mm cancellous BioOss, Osteohealth)/ 30 mL of PRP/ observation was after 11 months.	Platelet-rich-plasma did not make a significant difference in the production of vital bone in sinuses grafted with BioOss. The use of platelet-rich plasma with grafts consisting of 100% BioOss should be considered only in respect to the improved handling quality (containment) of the particulate graft material that can be achieved through the activation of the platelet-rich plasma with thrombin.
Aghaloo et al (2005)	15 white male rabbits with four 8mm diameter defects in cranium with a trephine bur with copious irrigation	grafted with freeze-dried mineralized bone (FMB) alone, FMB+PRP, freeze-dried demineralized bone (FDDB) alone, and FDDB+PRP/ 1–1.5 cm3 of PRP/ 4 months period	Radiographically, FMB+PRP showed a tendency toward increased bone density over FMB alone, and FDDB+PRP showed a tendency toward increased bone density over FDDB alone. Histomorphometrically, FMB+PRP showed a tendency toward increased bone area over FMB alone at 1 and 4 months, and FDDB+PRP showed a tendency toward increased bone area over FDDB alone, at 1 and 2 months.
Dominiak & Mierzwa-Dudek (2005)	1 patient with multiple complex bone defects of teeth	PRP+ heterogenous bone graft Bio−Oss Spongiosa®/ 5–7 ml of PRP/ 4 months period	After surgery, there was no presence of plaque and gingival bleeding. The average depth of periodontal pockets in four measurement points decreased by 10.6 mm and the average level of the loss of connective tissue attachment decreased by 11.4 mm. A pantomogram showed the reconstruction of the alveolar process bone tissue.
Shayesteh et al. (2005)	8 white rabbits with three identical full thickness bony defects with a round bur (3×6 mm) in the frontal and parietal bones with a distance of approximately 2mm from the sagittal and coronal sutures.	The defects was grafted with Deproteinized Bovine Bone Mineral (DBBM) and PRP+DBBM/ 0.5 ml of PRP/ 12 weeks period	A significant increase in bone formation was seen with the addition of PRP to DBBM at 2, 4 and 8 week intervals. At 12 weeks, the level of bone formation was similar between the two groups. There was also a significant increase in the rate of biodegradation of the DBBM particles with the addition of PRP at 2, 4, 8 and 1 weeks.
Steigmann & Garg (2005)	20 patients who required sinus augmentation with a crestal bone height of approximately 7–9 mm in the posterior maxilla	autologous PRP	PRP alone in appropriate sinuslift cases can produce bone growth
Casati et al. (2006)	10 male adult mongrel dogs with defects in dehiscence-type bone around dental implants. Before implant placement, one dehiscence-type defect (4 mm x5 mm) was bilaterally created on the buccal aspect of the implant osteotomies with a high rotation conic bur with sterile continuous saline solution irrigation. Two 4 mm x8.5 mm machined screw-shaped commercial pure titanium implants were placed on each side of the mandible, and the soft tissues were repositioned and sutured.	PRP gel/ 5 ml of blood were drawn from each dog, this blood was used to obtain separation of basic blood fractions (PRP)/ platelet count of PRP was 460,350 platelets/ml/ 3 months period	Bone-to-implant contact (BIC), bone density (BD) within the limits of implant threads, bone density (BO) and new bone area (NB) in a zone lateral to the implant, corresponding to bone defects, were obtained and measured. No statistically significant differences for any of the investigated parameters when PRP was used. Within the limits of the present study, it was concluded that platelet-rich plasma alone did not enhance bone regeneration for peri-implant defects
Czuryszkiewicz-Cyrana et al. (2006)	26 patients with diagnosed chronic and advanced periodontitis.	PRP, PRP + autogenous bone/ 8.5 ml of blood were drawn from each patient, this blood was used to obtain separation of basic blood fractions (PRP)/ observation after implantation of autogenous bone with	Autogenous bone with added PRP in treatment ofintrabony defects caused by periodontitis have given significant clinical improvement of the periodontal tissues. The combination of PRP and autogenous bone caused the elimination of a convenient environment for subgingival bacterial plaque eliminating periodontitis.

		added PRP was 12 months after surgery	
Kaul et al. (2006)	14 female rabbits with a cylindrical osteochondral defect in the patellar groove of each, with a manual cannulated burr (3.2 mm in diameter)	Lapine articular chondrocytes overexpressing a lacZ or a human FGF-2 gene sequence were encapsulated in alginate and further characterized. The resulting lacZ or FGF-2 spheres were applied to cartilage defects in the knee joints of rabbits/ 14 weeks period.	In vitro, bioactive FGF-2 was secreted, leading to a significant increase in the cell numbers in FGF-2 spheres. In vivo, FGF-2 continued to be expressed for at least 3 weeks without leading to differences in FGF-2 concentrations in the synovial fluid between treatment groups. Histological analysis revealed no adverse pathologic effects on the synovial membrane at any time point. FGF-2 gene transfer enhanced type II collage expression the cell morphology and architecture of the new tissue. Overall articular cartilage repair was significantly improved at both time points in vivo.
Klongnoi et al. (2006)	16 minipigs with extraction of maxillary premolars	Sinus augmentations were performed bilaterally using grafting materials: autogenous bone and Algipores® with or without PRP/ 5ml of PRP was available for each augmentation site/ 12 months period	The grafting materials chosen showed increasing levels of bone-implant-contact (BIC) and newly formed bone throughout the period of observation in both PRP and non-PRP groups. Adding PRP resulted in lower BIC and newly formed bone compared with autogenous bone grafts or Algipores alone. However, a statistical significance was not found. The percentages of the remaining bone substitute in both the PRP and non-PRP groups were closely comparable in all observation periods.
Mishra. & Pavelko (2006)	20 patients with elbow epicondylar pain for 15 months. All patients were considering surgery	injection of PRP (active group, n=15) or bupivacaine (control group, n=5)/ 2 to 3 mL PRP / observation was after 1. 3. 6 months and after 1 year	Eight weeks after the treatment, the PRP patients noted 60% improvement in their visual analog pain scores vs. 16% in control patients. At 6 months, the patients treated with PRP noted 81% improvement in their visual analog pain scores. At final follow-up the platelet-rich plasma patients reported 93% reduction in pain compared with before the treatment.
Sarkar et al. (2006)	16 sheep with critical diaphyseal long bone defect (critical size defect -2.5 cm)	autogenous PRP in a collagen carrier or with collagen alone (controls)/ 3.5 ml of PRP/ treatment extended for 12 weeks	Bone volume, mineral density, mechanical rigidity and histology of the newly formed bone in the defect did not differ significantly between the PRP treated and the control group, and no effect of PRP upon bone formation was observed; PRP could not promote bone regeneration in critical size defects with a low regenerative capacity
Plachokova et al. (2007)	45 rats, in which one cranial defect with a diameter of 6.2 mm	The defects were filled with combination with dense biphasic hydroxyl apatite (HA)/b-tricalcium phosphate (TCP) particles HA/b-TCP particles and HA/b-TCP particles combined with PRP gel/ 150 µl of PRP/ observation was after 1 and 2 weeks	A 6.2mm cranial defect is not a critical-sized defect in rats. Rat PRP had no effect on the early stages of bone healing in addition to an osteoconductive material. Dense HA/b-TCP particles showed a beneficial effect on bone formation already after 1 and 2 weeks of implantation in non-critical-sized cranial defects in rats.
Sánchez et al. (2007)	12 athletes with spontaneous complete rupture of the Achilles tendon	Control group (6 athletes not receiving PRGF), and test group (6 athletes receiving PRGF) PRGF/ 4 ml of PRGF/ 12 months period	Athletes receiving PRGF recovered their range of motion earlier, showed no wound complication, and took less time to take up gentle running and to resume training activities
Tunç et al. (2007)	22 patients with infrabony defects	10 ml of blood were drawn from patients, this blood was used to obtain separation of basic blood fractions (PRP)/PRP + DFDBA (demineralized freeze-dried bone allograft)	The results indicate that DFDBA/PRP combination is more effective than PRP alone for the treatment of infrabony defects, and the amount of CAL gain, PPD reduction, and bone fill increases when the infrabony defect is narrow and deep before DFDBA/PRP combination treatment.
Azzena et al. (2008)	75-year-old woman with painful, adherent scar at the shoulder level	20 ml of blood were drawn from patient, this blood was used to obtain separation of basic blood fractions (PRP)+fat	The results were satisfactory, showing fat survival 1 year after surgery.
Cieslik–Bielecka A. et al. (2008)	with influence of PRG on healing of mandibular odontogenic cysts	cases divided into control (no PRG treatment) and experimental (PRG-treated) groups; each participant was followed on a regular basis with clinical examinations, roentgenograms, and dual-energy x-ray absorptiometry (DEXA) examinations/ PRG-gel	Clinical observations showed that oral mucosa healed faster in patients treated with PRG compared with cases where gel was not added. Roentgenograms and DEXA examinations showed considerable enhancement of bone regeneration beginning from the 5th week and continuing during subsequent periods after implantation of PRG in the experimental group compared with the control group.
Gawande & Halli (2008)	20 patients having bilateral mandibular third molar impaction with similar angulations	5 ml of blood were drawn from patients, this blood was used to obtain separation of basic blood fractions (PRP)/ PRP gel	The result of the study shows rapid bone regeneration in the extraction socket treated with PRP when compared with the socket without PRP. Also there was less postoperative discomfort on the PRP treated side.
Simman et al.	48 rats with following	thrombin-activated PRP	Radiographic analysis demonstrated higher callus

(2008)	creation of unilateral open femur fractures	(PRP treated group) and saline (control group)/ 500µl of PRP/ 4 weeks period	to cortex in the PRP group. Three-point load bearing showed increased bone strength following PRP treatment. Fracture histology showed enhanced bone formation in the PRP group. Immunohistochemistry and Western-blotting demonstrated healing-associated changes in transforming growth factor (TGF)-β1 and bone morphogenetic protein (BMP)-2.
Lyras et al. (2009)	40 skeletally white rabbits. Two groups 10 rabbits each (PRP and control group) were used to evaluate mechanical properties and histology after 14 days and two groups ten rabbits each (PRP and control groups) were used to evaluate mechanical properties and histology after 28 days. The 3-mm width of the defect is approximately equal to one-third of the width of the tendon	PRP-gel/ 8 ml of blood were drawn from each dog, this blood was used to obtain separation of basic blood fractions (PRP)/ 28 weeks period	The mechanical properties of the regenerated tendon in the PRP group were significantly improved in relation to the control group. It appears that PRP has a strong effect in the early phase of tendon healing. This effect is probably due to the growth factors that are released from the platelets during activation
Pieri et al. (2009)	8 adult minipigs in which two mandibular premolar teeth were extracted bilaterally; the defects of 3.5 mm diameter and 8 mm depth were created in each root site	The defects were randomly grafted with autogenous mandibular bone, (fluorohydroxyapatite) FHA alone, PRP-FHA, or mesenchymal stem cells	MSCs-PRP-FHA-treated sites showed new vital bone between residual grafting particles. PRP-FHA- and FHA-treated sites showed residual particles in a background of marrow soft tissue with a moderate quantity of newly formed bone. Autogenous bone (46.97%) and MSCs-PRP-FHA (45.28%) produced a significantly higher amount of vital bone than PRP-FHA (37.95%), or FHA
		MSCs-PRP-FHA/ 16 mL of PRP gel/ observation was after 3 months	alone (36.03%). Further, the MSCs-PRP-FHA-treated defects showed a significantly higher percentage of contact between graft particles and newly formed bone compared with PRP-FHA and FHA group (59.23% vs. 48.37% and 46.43%, respectively).
Spyridakis et al. (2009)	52 patients with pilonidal sinus disease who underwent open excision and secondary closure of the surgical wound (n=22) or additional local postoperative infusion of platelet-derived growth factors (n=30) were evaluated. Duration of total wound healing and time to return to normal activities were evaluated.	PRP-gel/ 4 weeks period	Wound-healing rates were much greater for the platelet group. Complete healing of the surgical wound required 24 days for the PRP-gel, more than 30 days for the control group. PRP-gel group returned to their normal activities around the postoperative day 17 vs. control group day 25. The use of platelet-derived growth factors directly to the surgical wound enhances the healing process resulting in faster recovery of patients surgically treated for pilonidal sinus disease.
Szypuła & Kędziora (2009)	10 patients with chronic suppurative osteomyelitis resistant to surgery and antibiotics	PRP/ treatment duration dependent on the case	Use of a platelet rich plasma preparation in cases of posttraumatic suppurative osteomyelitis has a beneficial effect on healing and regeneration of bone.
Arenaz-Búa et al. (2010)	82 patients with bilateral impacted mandibular third molars	PRP, AUTOLOGOUS BONE +PRP, PRP +NOVABONE , PRP + DBX (DBX®-Allogeneic demineralized bone matrix NOVABONE®-synthetic material based on synthetic	Did not observe that the platelet-rich plasma accelerated bone formation in post-extraction sockets. Platelet-rich plasma mixed with other biomaterials facilitates the manipulation of the graft (made of hydroxyapatite, for example) and therefore could be useful as a biological carrier in
		calcium hydroxyapatite)	mandibular bone reconstruction due to its low cost and ready availability.
Filardo et al. (2010)	15 patients affected by chronic jumper's knee, with exercise-associated pain, pain or tenderness on palpation and imaging findings of degenerative changes and control group of 16 patients treated with the physiotherapy	PRP/ 20 ml of PRP/ injections were performed every 15 days, end of the treatment and at 6 months follow-up	Analysis showed a significant improvement in both the score recorded by an individual for their current health-related quality of life state and sport activity; decrease in the pain level on the from basal evaluation to the end of to the end of the PRP injections.
Hartmann et al. (2010)	15 patients, who had suffered an injury of the thoracic or lumbar spine and had undergone an anterior fusion using cages/ 20 patient in control group	Bone graft combined with PRP. A control group made up of 20 patients received a similar treatment, but without the addition of PRP/ 110 ml of blood were drawn from each dog, this blood was used to obtain separation of basic blood fractions (PRP).	In both groups, 40% of the patients had reached a complete fusion in the CT scans. No or minimal fusion was documented in 20% of the PRP group and 30% of the control group. When measuring the density within the newly formed bone mass, both groups showed nearly identical percentages with a density of over 100 Hounsfield units (HU). The share of bone with a density of over +500 HU was 29.33% in the PRP group and 23.57% in the control group. Within the partition of over +100 HU, the absolute density was significantly higher in the PRP group.
Khoshzaban et al. (2010)	30 male rats with the cavity which was drill in the Calvarias 6.2 mm in diameter	Group1:treated by PRP/Bio-Oss; group 2:treated by PRP-Gel /Bio-Oss; group 3:had two cavities; Bone chips mixed with individual blood/ 4-5 ml of blood were drawn	The author suggested that neither PRP nor PRP-Gel could be as beneficial as bone chips. Statistically, in PRP-Gel group, due to the existence of fibrin and thrombin, solid bone bridging at the treated site was indicated.

		from each rat, this blood was used to obtain separation of basic blood fractions (PRP)/ 16 weeks period	
Kon et al. (2010)	100 consecutive patients, affected by chronic degenerative condition of the knee	PRP/ 5 ml of PRP/ 12 months period	The preliminary results indicate that the treatment with PRP injections is safe and has the potential to reduce pain and improve knee function and quality of live in younger patients with low degree of articular degeneration.
Luaces-Rey et al. (2010)	20 patients who underwent secondary bone graft in the alveolar cleft	autogenous bone graft, autogenous bone and platelet-rich plasma (PRP)/ 6 months period	No significant differences were found between both therapeutic groups on bone regeneration.
Lyras et al. (2010)	48 white rabbits with a full thickness defect in the central portion of the patellar tendon	PRP gel was then applied and filled the tendon defect (in the control group, without the application of PRP)/ 2 ml of PRP/ 4 weeks period	The histological examination showed a superior healing process in the PRP vs. control group; the tissue formed in the PRP group was more mature and dense with less elastic fibers remaining. Neovascularisation was significantly higher in the PRP group.
Mallo et al. (2010)	1 patient with shoulder pain in his right dominant extremity. His pain was exacerbated with overhead movement, especially forward elevation above 90°. The patient had pain with passive forward lexion at 100° as well as marked pain with passive forward flexion to 90° combined with internal rotation. He showed no tenderness to palpation over his biceps tendon.	Diagnostic arthroscopy with partial bursectomy, subacromial, and PRGF injection at the conclusion of the surgery/ 3ml of the PRGF was delivered into subacromial space/ 9 months period	The patient achieved active and passive forward flexion from 0° to 160° with external rotation from 0° to 70° at the end of 3 months, he reported persistence and progressive worsening of his preoperative pain. An exuberant synovitis subsequently developed, maybe resulted from the PRGF treatment.
Sun et al (2010)	48 rabbits with osteochondral Defects in the femoropatellar groove were left untreated, treated with autogenous PRP in a polylactic-glycolic acid (PLGA), or with PLGA alone	A loading volume consisting of 20 µl human lyophilized thrombin and 80 µl of PRP mixture was sequentially pipette loaded onto each PLGA scaffold placed in a well of a 24-well plate/ 80 µl of PRP/ 12 weeks period	PRP yielded better osteochondral formation than the empty PLGA scaffold after 12 weeks. PRP incorporated in PLGA could successfully resurface the defect with cartilage and restore the subchondral bone in the rabbit model.
Alpigiani et al. (2011)	1 patient with right hip pain and functional limitation. Of the hip joints which showed osteonecrosis in chondral/ subchondral regions at the superior-external convexity of the right femoral head	Bone Marrow Cells (BMC) added to PRP. Implanted BMC plus PRP in the osteonecrotic region with improvement of pain and mobilization.	Patient walks autonomously, without joint pain and with improved hip movements. BMC plus PRP implantation represents only a palliative care to delay surgical treatment or if it is a valid alternative to traditional orthopedic surgery
Cervellin M. et al. (2011)	Forty young athletes with the indication of ACL reconstruction with patellar tendon grafts	56 ml of blood were drawn from patients, this blood was used to obtain separation of basic blood fractions (PRP)/ PRP gel	In 85% of PRP group patients, the tibial and patellar bone defect was satisfactorily filled by new bony tissue (70% of bone gap filled), whereas this percentage was just 60% in control group patients.
Dalal et al. (2011)	1 patient complained of swelling in upper right front tooth region. There was a single, diffuse swelling with smooth overlying skin, extending antero-posteriorly from right of nose to the lateral canthus of eye and superior-inferiorly from right ala of nose to right commisure of lip. Oval swelling of size 2×1cm, with normal overlying mucosa. Patient was operated under general anaesthesia and enucleation of cyst.	PRP gel+ autologous bone graft/ 10 ml of blood were drawn from patient, this blood was used to obtain separation of basic blood fractions (PRP)/ 3 months period	Promising result with fast and good quality bony regeneration was observed.
Dhollander et al. (2011)	5 patients with focal cartilage defects involving the patella and with clinical symptoms (pain, swelling, locking and ''giving away'')	autologous matrix-induced chondrogenesis (AMIC)+PRP gel	A clinical improvement became apparent after 24 months of follow-up. The formation of intralesional osteophytes was observed in 3 of the 5 patients during the 2 years of follow-up.
Martins et al. (2011)	22 patients with cancer and bisphosphonate-related osteonecrosis of the jaws	PRP + LPT(laser phototherapy)/ 4ml of PRP/ period 6 months	A significantly higher percentage of patients reached the current state of bisphosphonate-related osteonecrosis of the jaws (BRONJ) without bone exposure (86%) in the PPR plus LPT group than in the pharmacological (0%) and surgical (40%) groups after 1-month follow-up assessment. These results suggest that the association of pharmacological therapy and surgical therapy with PRP plus LPT significantly improves BRONJ healing in oncologic patients.

Orcajo et al. (2011)	1 patient with type 2 diabetes, insulin treated since 2002 with microangiopathy and diabetic Polyneuropathy. The patient had amputations of the 1st, 2nd and 3rd toes of right foot due to persistent ulcers. An infection in the 4th toe caused an ulcer with bone exposed, which finally resulted in amputation of the toe	PRGF/ 5 ml of PRGF in syringe which was connected to a sterile cannula and introduced into the mal perforant ulcer from the dorsum to the sole of the foot/ 10 weeks period	After PRGF treatment the severe mal perforant ulcer completely healed.
Rezaie et al. (2011)	20 white diabetic rabbits with hole in size of 4×5 mm in diameter and depth between lateral and medial condyles of left femur	autologous PRP gel/ 5 ml of blood were drawn from each rabbit, this blood was used to obtain separation of basic blood fractions (PRP)/ observation was after 2 months	PRP provide a rapid regeneration of bone defects in femoral cancellous bone in diabetic rabbits.
Sardari et al. (2011)	Five male, mixed breed dogs under general anesthesia (using acepromazine 0.05 mg/kg IM and ketamine 20 mg/kg IM, followed by halotan) had six full-thickness skin wounds (20×20 mm) created on the back of each dog symmetrically	Total amount of 1,5 ml of PRP given to dogs in 1, 3 and 5 day after wounding.	No significant difference was seen in the percentage of wound contraction, epithelialization, and healing between test and control. There were no significant differences between median of hydroxyproline levels between left and right wounds in dogs treated with dexamethasone. There were no significant differences between median of epithelialization, inflammatory
	(three wounds in each side)		cell infiltration, presence of dermal granulation tissue, fibroblast proliferation, arrangement of fibroblasts, collagen deposition, and collagen bundle formation scores, in the specimens of left and right wounds. The results of the study demonstrated that PRP did not have significant effects to promote cutaneous regeneration and wound healing in dogs treated with dexamethasone at least 16 days after last injection.
Shahabuei et al. (2011)	48 dogs with experimental defects with 5 mm horizontal and 5mm vertical open probing depth in furcation areas of each premolar	test group grafted with Bio-oss+PRP, control group grafted with bio-oss alone, negative control group in which no graft was used/ 3 months period	The bone fill was 61% in the test group, 58 % in the control group, and 39 % in the negative control group. In the control group, the lamellar bone formation was higher and the chronic inflammatory infiltration was lower.
Zhang et al. (2011)	27 white rabbits with the unilateral 15-mm critical-size defects in the distal radial diaphysis	The bone defect was filled with BG (degradable bioactive borate glass) alone, BG combined with autologous PRP or left empty/ 0.8 mL of PRP/ treatment extended for 12 weeks.	At 12 weeks, the volume of the newly formed bone in the BG + PRP animals was significantly higher than in those receiving only BG; PRP improved bone healing in a diaphyseal rabbit model on BG. The combination of PRP and BG may be an effective approach to repair critical defects.

Wright et al. and Sanchez et al. showed that the use of PRP is an effective method of treatment in muscle injuries; the use of PRP accelerated recovery of patients and professional athletes who were given platelet concentrates (51, 52).

Also the first clinical study carried out on humans with PRP, used for the treatment of tennis elbow, provided the basis for consideration of platelet-rich plasma as an alternative to surgical treatment in patients with chronic tendinosis resistant to conservative therapies (53).

Man et al. (2001) used PRP in patients undergoing cosmetic surgery, including face lifts, breast augmentations, breast reductions and neck lifts, and reported that bleeding capillaries were effectively sealed within 3 minutes after application of the platelet gel (PRP) and fibrin glue (PPP) (54).

Recently, there has been research and tests on the use of PRP in order to obtain an easy-to-handle biologic gel that could be mixed with adipocytes. It is postulated that proteins from PRP could stimulate revascularization of the implanted adipocyte rich gel and constitute a three-dimensional matrix that allows for the arrangement of adipocytes into a correct spatial organization (55). Cervelli et al. treated patients affected by facial aging, characterized by atrophy of the subcutaneous tissue and deficits of soft tissue with loss of volume and elasticity with loss of volume and elasticity, with PRP mixed with centrifuged fat tissue (56).

In particular, growth factors regulate cellular events in wound healing, such as proliferation, differentiation, chemotaxis and morphogenesis of tissues and organs, acting in an autocrine, paracrine or endocrine manner. They are deposited in the extracellular matrix and are then released during matrix degradation. Their interaction with surface receptors on the target cells activates an intracellular signalling pathway that induces transcription of the

messenger RNA and proteins needed for the regenerative process. These growth factors, in combination with other transcription factors, then activate a set of genes. The growth factors also induce specific changes at a cellular level. All of these effects are controlled by feedback mechanisms involving binding proteins and other growth factors. Growth factors may also interact with one another, consequently forming a cascade of different signal proteins with multiple pathways, ultimately leading to the activation of gene expression and then protein production. Even in a single cell type, the nature of growth factor action may depend on the context set by other substances present. For example, TGF- stimulates growth of certain fibroblasts in vitro in the presence of PDGF but inhibits their growth if epidermal growth factor is present (21).

Giannobile et al. evaluated the interactions of IGF-I with other growth factors, including PDGF and TGF-b. They demonstrated various degrees of mitogenic activity, and the increase in DNA synthesis receiving IGF-I/TGF-b1 and IGF-I/PDGF-BB treatment was significantly greater than the individual factor (57). Mott et al. examined the effects on proliferation of a combination of IGF-I/TGF-b1, PDGF-BB/TGF-b1 and IGF-I/PDGF-BB, and showed that the proliferation of osteoblasts was enhanced by a combination of growth factors (58).

It is worth noticing that there are also some studies that do not suggest PRP as an effective factor for bone reconstruction (59, 60, 61). From a therapeutic point of view, there are two major processes related to the potential of this technology: the release of proteins and growth factors from platelets that actively stimulate tissue regeneration, and the formation of a three-dimensional fibrin matrix that retains and later releases part of the growth factors, and which also acts as a temporal nesting scaffold for the cells. After activation, platelets release a pool of biologically active proteins and other substances that are able to influence the recruitment, growth and morphogenesis of cells. The creation of a chemotactic gradient would mediate cell recruitment and the onset of the healing process. PRGF causes a fourfold increase in cell growth in vivo 72h after its application, enhances collagen deposition (twofold at 7 days) and while stimulating the proliferation of human gingival fibroblasts (twofold), inhibits keratinocyte growth by 40% (62, 63). Initiation of bone cell growth takes place mainly with the participation of growth factors PDGF, TGF-b and IGF. Their main source is platelet rich plasma (PRP) which is used among others as a component of platelet gel. In order to fully exploit the regenerative properties of PRP, it is combined with osteoconductive material, resulting in a demineralized bone matrix used to regenerate bone defects (64).

PDGF (Platelet-derived growth factor) seems to have numerous positive effects on wound healing including mitogenesis, angiogenesis, activation of macrophages and upregulation of other growth factors. PDGF is a powerful mitogen for fibroblasts and smooth muscle cells and is involved in all three phases of wound healing, including angiogenesis, formation of fibrous tissue, and re-epithelilization (65). PDGF is known to emerge from degranulating platelets at the time of injury. There are four possible isoforms of this thermo-resistant dimeric glycoprotein (molecular weight of approximately 30 kDa) with 3 disulphide bonded polypeptides referred to as A, B and C chains: AA, BB, CC and the heterodimeric AB. However, more recently an additional isomer PDGF-DD has also been verified (66). In lesser quantities, A-A and B-B homodimers exist in human beings with the same activity. Cells susceptible to PDGF action express α (or A-type receptors) and β (or B-type receptors), which are both involved in the transduction of mitogenic stimuli, while the β receptor mediates chemotactic stimulation. PDGF alpha-receptors bind all isoforms with high affinities; however, beta-receptors bind PDGF-BB with high affinity and PDGF-AB with a comparatively lower affinity, but do not bind PDGF-AA with any considerable affinity (67, 68).

Its mechanism is to activate cell membrane receptors on target cells, which in turn are thought to develop high-energy phosphate bonds on internal cytoplasmic signal proteins; the bonds then activate signal proteins to initiate a specific activity within the target cell (6).

All isoforms have proliferative activity on periodontal ligament fibroblasts, the AA and BB isoforms enhance proliferation of bone cells increasing the production of PDGF-AA in osteoblast cultures by an autocrine process. PDGF is also chemotactic for adipocytes, and it promotes collagen type I and protein synthesis, stimulates cell growth, but also changes cell shape and motility. It is the most thoroughly described growth factor in terms of its effects on the periodontium both in vitro and in vivo. The PDGF stimulates mitogenesis of the marrow stem cells and endosteal osteoblasts transferred in the graft to increase their numbers by several orders of magnitude. It also begins an angiogenesis of capillary budding into the graft by inducing endothelial cell mitosis and additionally indirectly, by activating macrophages (69, 70).

TGF-βs (Transforming Growth Factor beta) this superfamily of growth and differentiating factors, including among others, BMP (Bone Morphogenetic Protein), TGF-β1, TGF-β2 (with molecular weights of approximately 25 kDa) are synthesized and found in platelets and macrophages, as well as in some other cell types, and are involved with bone regeneration in mitogenesis of osteoblast precursors, acting as paracrine growth factors. The paracrine mechanism also causes the

aforementioned target cells to release proteins of TGF-β, acting on the cells in the direct vicinity. The activation process is maintained by an autocrine mechanism, involving the effect of growth factors secreted by the cell on its own membrane receptors. When released by platelets or secreted by macrophages, TGF-β exerts its effects on adjacent cells, including fibroblasts, marrow stem cells, endothelial cells, and preosteoblasts. TGF-β stimulates angiogenesis and chondrogenesis, the production of fibronectin, glycosaminoglycans, and collagen in connective tissue. In addition, it inhibits the proliferation of lymphocytes (71). Stimulates monocytes to secrete FGF, PDGF, TNF– α and Interleukin-1. TGF-β initially activates fibroblasts and preosteoblasts to mitose and increase their numbers, as well as promoting their differentiation toward mature functioning osteoblasts. Continued TGF-β secretion influences osteoblasts to lay down bone matrix and the fibroblasts to lay down collagen matrix to support capillary ingrowth (6). Fibroblasts under the influence of TGF-β increase the synthesis of collagen, fibronectin, and integrin. TGF-β also inhibits the enzymes that degrade these proteins (72). Additionally, they inhibit osteoblast formation and the proliferation of epithelial cells in the presence of other growth factors (73). TGF-β therefore represents a mechanism for sustaining a long-term healing and bone regeneration module, and even evolve into a bone remodeling factor over time (6). Repeated injections of TGF-β resulted in ossification by means of endochondral bone formation in the long bones (74). In addition, it promotes angiogenesis and extracellular matrix production. TGF-β chemotactic factor stimulates proliferation and differentiation of mesenchymal stem cells and promotes the synthesis of collagen type I by osteoblasts, and has been reported to be angiogenic (75).

IGF-1 (Insulin-like growth factor; somatomedin-C) is thought to increase the number of osteoblasts and thereby accelerate bone formation, the formation of bone matrix by promoting pre-osteoblast proliferation and stimulates the synthesis of osteocalcin, alkaline phosphatase activity in osteoblastic cells and collagen type I biosynthesis by osteoblasts and prostaglandin E2 in fibroblasts (76). It also stimulates the proliferation and differentiation of mesenchymal stem cells in chondrogenesis, adipogenesis, myogenesis, promotes neuronal differentiation and induces a chemotactic effect on vascular endothelial cells (77, 78). IGF has 2 forms, I and II, each of which has 2 single chain peptides, with respective molecular masses of 7.7 and 7.5 kDa. IGF binds to the same receptors as insulin and is involved in the development of many tissues, including the teeth. Both forms of IGF are potent factors for the survival of hematopoietic cells, fibroblasts and the nervous system. IGF is also an important modulator of cell apoptosis, and in combination with PDGF, can promote bone regeneration. IGF-I may directly stimulate the cells as an autocrine factor, additionally IGF-I transcripts have been isolated from macrophages in wounds, suggesting that this growth factor may also act as a local messenger as a paracrine factor. The autocrine growth-promoting activity is inhibited by IL4 (21). IGF has an effect on intraosseus osteoblasts, intensifying the formation of bone trabeculae in the spongy bone transplant. Increased cell activity at the beginning is the effect of PDGF, TGF-ß and IGF and, to a smaller degree, other factors (79).

EGF (Epidermal growth factor) has a mitogenic and chemotactic effect on fibroblasts and epithelial cells. It was described as an inductor for cell migration and stimulator for the formation of granulation tissue. The cells which synthesize and express receptors for EGF are mainly fibroblasts, pre-osteoblasts and pre-chondrocytes, indicating the participation of this growth factor in physiological processes connected with those cells (80). Menetrey et al. proved that FGF and IGF-1 accelerate the muscle healing process (81).

b-FGF (Basic-fibroblastic growth factor) is a member of the multifunctional fibroblast growth factor family. FGFs are pleiotropic factors that act on various cells including endothelial cells. The biological activity of FGF-2 is mediated through a dual receptor system consisting of four high-affinity, tyrosine kinase receptors and low-affinity, heparan sulfate proteoglycans located at the cell surface. To date, the FGF family consists of 23 members, all of which contain a conserved 120 amino acid core region that contains six identical, interspersed amino acids. The biological effect of FGFs is mediated by four tyrosine kinase FGF receptors, with multiple specificities noted for almost all FGFs (82). Basic fibroblast growth factor (FGF-2), present in platelet α-granules, stimulates and coordinates the mitogenesis of mesenchymal stem cells during growth, maintenance and tissue repair. Such effects were reported for fibroblasts, osteoblasts, chondrocytes, smooth muscle cells and skeletal myoblasts. FGF-2 stimulates chemotactic migration of marrow-derived mesenchymal cells into articular cartilage defects, resulting in a higher cell density. Angiogenesis is also enhanced through endothelial cell stimulation to undergo mitosis and migration (83). Analysis of type II collagen expression suggests that FGF-2 may selectively increase type II collagen content. In contrast, FGF-2 did not lead to an increase in type I collagen production (84).

VEGF (Vascular Endothelial Growth Factor) is essential for many angiogenic processes both in normal and pathological conditions. It is a thermo-resistant growth factor, a protein with a mass of 40-45 kDa, which induces the chemotaxis and proliferation of endothelial cells to provoke the angiogenesis and hyperpermeability

of blood vessels, and can promote healing of chronic wounds and aid in endochondral ossification. In vivo, application of VEGF to the surface of rat molars promoted cementogenesis after reimplantation. It is mitogenic, proapoptotic and promotes the chemotaxis and differentiation of epithelial, renal and glial cells as well as fibroblasts (85). VEGF has been successfully used in the treatment of various vascular diseases (86, 87).

PF4 (Platelet Factor 4) is a chemokine that functions as a negative regulator of angiogenesis and as a powerful inhibitor of endothelial cell proliferation. It is a chemotactant for neutrophils and fibroblasts and is a potent antiheparin agent (88).

Beside the most important GFs, described above, following mediator molecules are released from the platelet following activation: IL-8 (interleukin-8), TNF-α (tumour necrosis factor alpha), CTGR (connective tissue growth factor), GM-CSF (granulocyte macrophage colony stimulating factor), KGF (keratinocyte growth factor), and Ang-2 (angiopoetin).

The results of clinical applications using different kind of rich platelets preparations are presented in Table 1.

The role of platelet growth factors in angiogenesis

In recent years we have seen a growing interest in the process of neovascularization from existing structures (angiogenesis is the formation of new blood vessels from preexisting vasculature), both in relation to therapeutic angiogenesis, neovascularization in ischemic diseases and the search for inhibitors of tumor vasculature. It is a process essential for wound healing, and also in the formation of blood vessels in various disease processes such as atherosclerosis and cancer (89, 90).

In the process of angiogenesis, a key role is played by migrating and proliferating endothelial cells (EC), with paracrine interaction of growth factors the most important stimulating factor. Endothelial cell proliferation is very slow and the length of their turnover rate exceeds 1000 days (91), but it is an easily excitable structure, responding quickly to stimuli which activate it and change its function. Angiogenesis is the result of a complex interplay between growth factors, vascular endothelial cells, extracellular matrix molecules, chemokines and cell signalling molecules. The process of angiogenesis begins with the decomposition of the basement membrane, allowing the migration and proliferation of endothelial cells and their distribution in the extracellular matrix. It was recently demonstrated that platelets, as a cellular system, could induce an angiogenic response and that platelet microparticles affect EC, protecting them from apoptosis and inducing proliferation and formation of tubule-like structures (92, 93).

Among the growth factors two play a crucial role in angiogenesis, i.e. VEGF and bFGF; both are released by platelets and may be applied when using PRGF or PRP. These growth factors induce neovascularisation and chemotaxis of fibroblasts and tenocytes and stimulate fibroblast and tenocyte proliferation and the synthesis of collagen (94, 95).

VEGF has been studied extensively and plays a critical role in both vasculogenesis and angiogenesis (96, 97, 98). The pro-angiogenic effect of VEGF is associated with the presence of receptors for this factor. VEGF binds to tyrosine-kinase receptors, Flt-1 (also known as VEGF receptor 1) and Flk-1 (also known as VEGF receptor 2, KDR as human counterpart), which are present on endothelial macrophages, monocytes and some tumor cells. Lymphatic endothelial cells have different receptors (99, 100). VEGF signalling is modulated by angiopoietin-1 (Ang1) that binds to receptor tyrosine kinases (Tie-2) (101). It has been shown to stimulate vascular bud formation and maintain endothelial cell survival. The action of Ang1 is associated with mesenchymal cells surrounding the endothelium. An excess of Ang1 causes the production of more numerous and finer capillaries (102). Ligand binding to Flk-1/KDR induces autophosphorylation of Flk-1/KDR intracellular tyrosine residues and activates several signaling pathways, leading to cell proliferation, survival, migration, and permeability. VEGF, binding to one of its receptors, promotes endothelial cell proliferation which otherwise shows limited growth potential in the absence of stimulation by this factor. Stimulation of the second receptor on mesodermal stem cells causes the transformation of these cells into endothelial cells (103). Recent evidence suggests that cyclooxygenase-2 (COX-2) modulates angiogenesis by interacting with the VEGF system. Nitric oxide (NO) is a vasodilator and is implicated in VEGF mediated vascular permeability and angiogenesis (104).

bFGF is a powerful mitogenic and chemotactic factor for endothelial and smooth muscle cells. Their action is dependent on interaction with the homeostatic factor – heparin. FGF interacts with heparinsulphate proteoglycans (HSPGs) and transmembrane receptor tyrosine kinase FGFR (FGFR-1, FGFR-2, FGFR-3, FGFR-4). Upon binding with their angiogenic ligands (FGF-1, FGF-2 and FGF-4), FGFRs are autophosphorylated leading to activation of several intracellular signalling pathways leading to the recruitment of Shc, FRS2 and Crk adaptor molecules (105). It is perhaps the interaction with HSPG that makes extracellular matrix (ECM) a key player in the regulation of angiogenesis. Induction of endothelial cell proliferation by FGF leading to angiogenesis, involves not only the activation of the mitogen-activated protein (MAP) kinase pathway, but also sustained activation of protein kinases C (PKC) and PI3 –kinase (106). Once the path for cell migration has been carved, FGF-1, FGF-

2, FGF8b isoform and FGF-10 promote chemotaxis in endothelial cells (107, 108). PI3-kinase plays a pivotal role in mediating EC survival, proliferation, cytoskeleton reorganization, and cell motility, all critically important for vessel growth. PI3-kinase is activated by VEGF and bFGF through VEGF receptor (Flk-1/KDR) on EC upon binding to its ligand (VEGF); FGF receptor-1; and via Src kinase family members (109). The platelet derived endothelial cell growth factor PD-ECGF is a monomer with a weight of 45 kDa, which stimulates the synthesis of DNA in endothelial cells but does not affect the proliferation of these cells (110).

Because platelets are the first cells to appear in wounds and inflammatory foci, PD-ECGF may prepare the vascular bed for changes caused by other growth factors (111, 112). One should remember that PRP may also be a source of factors that inhibit angiogenesis. Produced by platelets, platelet factor 4 cytokine of low molecular weight, inhibits the formation of new blood vessels due to blocking the binding of angiogenic growth factors to membrane proteoglycans. It can inhibit in vitro proliferation and migration of endothelial cells. Activity of endothelial cell mitogens, such as VEGF and bFGF, are sensitive to inhibition by platelet factor 4 (113).

Conclusion

Autogenous platelet-rich plasma (PRP) was first used in the 1990s and is raising interest and controversy among researchers and clinicians. Investigations relating to the use of autogenous material in medicine focus on inter-relationships between factors released during the activation of platelets, plasma factors, potential additive mechanisms, and the search for optimal preparation procedures and dosing of blood products, including PRP.

Additionally at this point is an increased focus on the cellular content, and the direct effects of the leukocytes and platelets themselves, not only on the growth factor synthesis and release, but also on the direct regulation of the inflammatory response, healing process, and anti-infectious activities of these preparations.

These technologies have been popularized over the last decade, particularly in cardiac surgery, in many areas of dentistry such as periodontics, oral implantology and oral and maxillofacial surgery, orthopedics, repositioning skin flaps in plastic surgery, against small diffuse bleedings, a mal perforant ulcer in the foot of a person with diabetes, to speed up the healing process of burns, to promote retinal neurogenesis, and in the treatment of musculoskeletal injuries and disorders but also in sports medicine.

PRP is mainly used in maxillofacial, restorative, and orthopedic surgery, as well as cardiology, implantology and cosmetic surgery. The pro-angiogenic mechanisms that occur with the participation of platelet grow factors are still not fully understood and require further research, both in vitro and in vivo.

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