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Review

# The use of platelet-rich plasma in bone reconstruction therapy

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ABSTRACT

The use of platelet-rich plasma (PRP) in bone reconstruction therapy was introduced in the late 1990s. Since then, many scientists and clinicians have employed it in orthopaedic and oral surgeries. Unfortunately, studies that analyze the use of PRP are somewhat controversial as some conclude that the use of PRP may favor bone regeneration and others conclude that the use of PRP is irrelevant. By listing and analyzing the biological effect that each factor released by the activated platelets can have in bone regeneration, the present review answers the question of why PRP may be useful in bone reconstruction therapy. Subsequently, by examining the studies that have both successfully and unsuccessfully utilized PRP, it suggests how PRP might be used in order to achieve successful results in orthopaedic and dental bone reconstruction surgeries.



## 1. Bone regeneration

Throughout the life of an individual, bone is subjected to microdamages that undergo regeneration or repair without giving rise to functional or anatomical defects. Differently, the regeneration or repair of the missing bone in large defects may be difficult to be accomplished without interventions such as bone grafting. Recent exciting discoveries in bone biology [1,2] have introduced novel therapeutic approaches for bone regeneration based on recombinant osteoinductive proteins. Clinical trials show the efficacy of recombinant human Bone Morphogenetic Protein 2 (rhBMP2) in bone regenerative therapy [3–5]. However, these trials also show that in cases of regeneration of large bone defects such as open tibial fractures there is still a need for surgical re-entry in approximately 50% of the cases treated with rhBMP2. The challenge of the large bone defect lies in designing devices and biomaterials that can foster the bone wound healing process into the appropriate pathway that leads toward the complete regeneration of the missing tissue. Novel strategies are still required to overcome this challenge.

A wound healing process is composed of three major phases: (1) the acute inflammatory phase, which includes platelet aggregation and activation and the migration of granulocytes and macrophages; (2) the mesenchymal cell proliferation and differentiation phase; and (3) the phase of regeneration of the missing tissue by tissue-specific cells [6]. Two possible pathways may follow the cascade of events initiated by the platelets: the repair pathway and the regeneration pathway. If the loss of tissue exceeds certain limits, the level of proliferation and differentiation of mesenchymal cells may not be sufficient for the regeneration of the tissue. In this case, non-mesenchymal cells, such as fibroblasts, proliferate and repair the injury with a scar (repair pathway). On the contrary, when the level of proliferation and differentiation of mesenchymal cells is sufficient, a complete regeneration of the missing tissue is achieved so that the correct function and activity of the tissue is fully re-established (regeneration pathway) [7]. Since platelets are the main regulators of the inflammatory phase and play an essential role in the proliferation and differentiation phase, scientists have proposed the delivery of a concentrate of platelets at the site of the injury as a successful strategy for fostering the regeneration pathway during bone wound healing. The concentrate of platelets is prepared *ex vivo* and is defined as platelet-rich plasma (PRP).

PRP is a concentrate of platelets in a small volume of plasma. Upon activation by an agonist, the platelets contained within the PRP release the contents of their granules consisting of inflammatory factors and growth factors. In 1998, Dr Robert E. Marx first proposed the use of PRP to enhance the initial phases of the bone wound healing [8]. Since then, PRP has been widely used in preclinical and clinical applications for regeneration of bone. However, despite the captivating rationale, its use has never produced consistent and reliable results in terms of bone regeneration. Several studies claim no positive effects of PRP for bone regeneration whereas other studies strongly advocate its use in bone regenerative therapy [9,10].

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In order to shed light on PRP as a novel mean for bone reconstruction therapy, this review investigates why and how its use may favor bone regeneration. By doing so, the controversial results of the scientific literature are evaluated and a guideline for the use of PRP in bone reconstruction therapy is proposed.

## 2. Why platelet-rich plasma can be helpful in bone reconstruction therapy

PRP is developed from autologous blood upon re-suspension of platelets in a low volume of plasma. Platelet count in PRP may vary according to the preparation technique, ranging from two to several fold above the physiological levels. Upon activation by an agonist, such as thrombin, the platelets contained in PRP release the following factors (in alphabetical order): ADP and ATP, Angiopoietin-2 (Ang-2), Connective Tissue-Activating Peptide III (CATP III), Epidermal Growth Factor (EGF), Factor V, Factor XI, Factor XIII, Fibrinogen, basic Fibroblast Growth Factor (bFGF of FGF2), Fibronectin, Insulin-like Growth Factor-I (IGF-I), Osteocalcin, P-Selectin (also called GMP-140), Platelet-derived endothelial cell growth factor (PDECGF or Thymidine phosphorylase), Platelet-derived Growth Factor (PDGF), Serotonin, Transforming Growth Factor-β1 (TGF-β1), Thrombospondin-1, Vascular Endothelial Growth Factor (VEGF), and Von Willebrand Factor (vWF) (see Table 1 for more details) [6,11–14].

Many of these platelets' factors have been shown to be involved in wound healing and in processes that culminate in parenchymal cell proliferation and tissue regeneration [6,11–14]. Thus, the possibility of delivering these matrix elements and growth factors within a bone defect is behind the theory of the use of PRP in bone reconstruction therapy. The explanation of the role of inflammation in bone regeneration goes beyond the scope of this review. This review will focus on the biological activity that each of these platelets' factors may have on bone competent cells and on bone regeneration in general (Table 1). This elucidation will lead to understanding why the use of PRP may favor bone regeneration.

### 2.1. ADP and ATP

Receptors for purines and pyrimidines are classified into two groups: P1 receptors with adenosine as the main ligand, and P2 receptors with ATP, ADP, UTP and UDP as the main ligands. Both osteoclasts and osteoblasts present P2 receptors [15]. Studies on rat osteoblast-like cells demonstrated that extracellular nucleotides interact with $P2Y_1$- and $P2Y_2$-like receptors [15] acting as mitogens for osteoblastic cells. Additionally, several studies have reported that nucleotides could act synergistically with growth factors such as Platelet-derived Growth Factor (PDGF) and Insulin-like Growth Factor (IGF) to induce osteoblast proliferation [15]. These synergistic activities suggest that, immediately after a bone injury, hormones and locally released growth factors may act together with ADP and ATP to induce bone formation. However, it was also shown that extracellular ADP, by activating $P2Y_1$ receptors on osteoclasts, is a potent stimulator of bone resorption. This action, though, is effective at nanomolar to low micromolar concentrations. No effects are evident at higher concentrations (20–200 μM) [15]. Also, ATP is a potent stimulator of the activation and formation of osteoclasts via the $P2X_2$ receptor [15]. Yet, the resorption effect of both ATP and ADP seem to be effective at low pH (~6.9) [16,17], which suggests that the activation of the ATP and ADP–dependent resorption pathways is acid dependent. Because PRP would deliver high concentrations of ADP and ATP at the wound healing site and because the acidic environment is present only at the initial stages of the bone wound healing process [8], one may expect that ADP and ATP would initially help with the bone remodeling of the functionally compromised bone and later would foster the bone regeneration pathway by inducing preosteoblast proliferation.

### 2.2. Angiopoietin-2 (Ang-2)

Angiopoietins comprise a family of growth factors acting on the vascular endothelium [18]. Four different angiopoietins (Angiopoietin-1, -2, -3, and -4) have been discovered that bind to a specific transmembrane tyrosine kinase receptor present on endothelial cells. Among them, and differently from the others, Angiopoietin-2 is likely to block the activity of this receptor under most circumstances. Thus, Angiopoietin-2 does not regulate endothelial cell proliferation but rather causes vessel destabilization and remodeling. Few studies have investigated the role that angiopoietin-2 may have in wound healing. One study [19] showed that Angiopoietin-2 expression was transiently upregulated during the formation of granulation tissue in normal mice whereas in diabetic mice the period of angiopoietin-2 upregulation was more extended. This study suggests that the impaired angiogenic response in diabetic animals could result from an imbalance in the levels of angiopoietins. However, the role that Angiopoietin-2 plays in blood clotting and in the subsequent cascade of events is still unclear. Yet, the vessel destabilization and remodeling induced by Angiopoietin-2 may be an important factor in the disruption of the damaged tissue before its regeneration. So far, no studies have investigated the effects that Angiopoietin-2 may have, directly or indirectly, on osteospecific cells and in bone regeneration in general.

### 2.3. Connective Tissue-Activating Peptide III (CATP III)

Connective tissue-activating peptide III (CTAP III) is a CXC proinflammatory chemokine derived from the chemokine precursor platelet basic protein (PBP) by proteolytic cleavage and has been identified in platelets, activated macrophages, neutrophils, T lymphocytes, and differentiating megakaryocytes [20,21]. CATP III was shown to have pleiotropic activities that include mitogenicity for connective tissue cells, induction of glucose uptake and glycosaminoglycan synthesis in fibroblasts, histamine release in basophils and induction of plasminogen activator activity. Other studies have also suggested that CATP III can support stem cell-derived haematopoiesis [22] but no studies were found on the direct effects that CATP III may have on osteoblasts and on bone regeneration.

### 2.4. Epidermal Growth Factor (EGF)

The Epidermal Growth Factor (EGF) family of mitogens comprises several members including EGF, transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), amphiregulin, epiregulin, betacellulin, neuregulins, the recently discovered epigen, as well as proteins encoded by *Vaccinia* virus and other poxviruses [18]. A series of experimental and clinical studies demonstrated a positive effect of EGF, TGF-α and HB-EGF on wound repair, suggesting that these endogenous growth factors are also involved in the healing process [23]. For instance, substantial levels of EGF and TGF-α were found in wound fluid from skin graft donor site wounds in patients with burn injuries [24]. However, little is known about the effects that EGF may have on bone tissue regeneration. One study shows that EGF at physiological dosage stimulates periosteal bone formation and increases endosteal bone resorption in the growing mouse [25]. Another study determined the role of EGFR signaling in endochondral ossification in EGFR-deficient mice. EGFR deficiency caused delayed primary ossification of the cartilage anlage and delayed osteoclast and osteoblast recruitment [26]. Although inconclusive, the presented literature

**Table 1**
List of the biologically active factors released by activated platelets, their general function and their specific activity on osteoblasts and bone regeneration.

| Biologically active factors released by activated platelets in PRP | | |
|---|---|---|
| Name | General function | Biological activity useful for regeneration of bone |
| ADP | Interacts with a family of ADP receptors found on platelets (P2Y1, P2Y12 and P2X1), leading to further platelet activation. Also, interacts with P2 receptors on osteoclasts and osteoblasts. | Bone remodeling and proliferation: Induces formation of inositol (1,4,5)-trisphosphate and transiently elevates $Ca^{2+}$ in osteoblastic cells. May act synergis tically with hormones and growth factors to induce bone regeneration. |
| ATP | Transports chemical energy within cells. ATP is also a signaling molecule and acts as agonist for blood cells. In fact, it interacts with P2 receptors on osteoclasts and osteoblasts. | Bone remodeling and proliferation: Induces formation of inositol (1,4,5)-trisphosphate and transiently elevates $Ca^{2+}$ in osteoblastic cells. May act synergistically with hormones and growth factors to induce bone regeneration. |
| Angiopoietin-2 (Ang-2) | During angiogenesis, it destabilizes the existing vessels. The gained induction of plasticity in the vessel environment is an essential step for the initiation of angiogenesis. | Vessels remodeling and angiogenesis. |
| Connective Tissue-Activating Peptide III (CATP III) | Member of the CXC chemokine family of proinflammatory mediators. It is involved in inflammatory reactions. | Inflammation. |
| Epidermal Growth Factor (EGF) | Plays an important role in the regulation of cell growth, proliferation and differentiation by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor. | Proliferation: EGF at physiological dosage stimulates periosteal bone formation and increases endosteal bone resorption. EGF receptor deficiency causes delayed primary ossification of the cartilage anlage and delayed osteoclast and osteoblast recruitment. |
| Factor V | In contrast to most other coagulation factors, it is not enzymatically active but functions as a cofactor. The activated factor X (FXa) enzyme requires $Ca^{++}$ and activated factor V to convert prothrombin to thrombin on the cell-surface membrane. This is considered part of the common pathway in the coagulation cascade. | Coagulation. |
| Factor XI | Like many other coagulation factors, it is a serine protease. It is activated into factor XIa by factor XIIa (FXIIa), thrombin, and it is also autocatalytic. FXI is a member of the "contact pathway" (intrinsic pathway) due to activation by FXIIa. | Coagulation. |
| Factor XIII | Enzyme of the blood coagulation system that crosslinks fibrin. Also called fibrin stabilizing factor. | Coagulation. |
| Fibrinogen | Soluble plasma glycoprotein mainly synthesized by the liver. Fibrin is made from its zymogen fibrinogen. | Coagulation. |
| basic Fibroblast Growth Factor (bFGF or FGF2) | It is present in basement membranes and in the subendothelial extracellular matrix of blood vessels. It mediates angiogenesis. | Proliferation and differentiation: By inducing proliferation and differentiation of preosteoblasts and osteoblasts is able to induce and sustain bone regeneration. |
| Fibronectin | High molecular weight glycoprotein. Binds to receptor proteins that span the cells membrane, called integrins. In addition to integrins, also binds extracellular matrix components such as collagen, fibrin and heparin. | Proliferation and differentiation: Integrin interactions with fibronectin are essential for osteoblast survival, proliferation, osteoblast specific gene expression, and bone matrix mineralization. Fibronectin and its derivative are being studied for applications in bone regenerative therapy. |
| Insulin-like Growth Factor-1 (IGF-1) | Play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis). It is required for achieving maximal growth during development. | Proliferation and differentiation: IGF-I stimulates osteoblastic cells in culture to proliferate and to synthesize bone matrix proteins and stimulates mRNA expression for alkaline phosphatase, osteopontin and osteocalcin in bone marrow stromal. |
| Osteocalcin | Non-collagenous protein found in bone and dentin. It plays a role in regulation of mineralization and calcium ion homeostasis. | Osteocalcin may act either as a cytokine or as a chemoattractant for osteoblasts and osteoclasts. However, no studies were found on the beneficial effects that osteocalcin may have in bone regenerative therapy. |
| P-Selectin (GMP-140) | Cell adhesion molecule (CAM) found in endothelial cells and activated platelets. | Coagulation. |
| Platelet-derived Endothelial Cell Growth Factor (PDECGF or Thymidine phosphorylase) | Protects cells from apoptosis and helps cell survival by stimulating nucleoside metabolism and angiogenesis. It is a nucleoside metabolism enzyme associated with the maintenance of healthy mitochondria and the recovery of cells from pathological stress. | Angiogenesis. |
| Platelet-derived Growth Factor (PDGF) | Plays a role in embryonic development, cell proliferation, cell migration, and angiogenesis. | Proliferation: Primary effect of PDGF in bone is related to its mitogenic activity. Controlled release of PDGF is beneficial to bone regeneration when applied in wound sites. |

**Table 1** (*continued*)

| Biologically active factors released by activated platelets in PRP | | |
|---|---|---|
| Name | General function | Biological activity useful for regeneration of bone |
| Serotonin | It is not only a neurotransmitter but also a potent mitogen that modulates the remodeling of various tissues. | Proliferation: Osteoblasts express serotonin receptors and serotonin is able to induce proliferation of osteoblasts-like cells (MC3T3-E1) *in vitro*. In serotonin transporter null mice (5-HTT (−/−)), cancellous bone volume in the lumbar vertebrae is reduced. |
| Transforming Growth Factor-β1 (TGF-β1) | Performs many cellular functions, including: cell growth, cell proliferation, cell differentiation and apoptosis. | Proliferation: TGF-β1 acts as paracrine and autocrineproliferative growth factor, affecting mainly fibroblasts, marrow stem cells and the preosteoblasts. |
| Thrombospondin-1 | Multifunctional protein that regulates the proliferation of smooth muscle cells and inhibits the proliferation and migration of endothelial cells. | Proliferation and angiogenesis: Although there are no studies that show a direct effect of TSP1 on osteoblasts or on bone regeneration, the multiple roles that TSP1 presents as ECM protein (including the activation of TGF-β1) leads to the importance that this molecule may have in the regulation of bone regeneration through the regulation of angiogenesis and cell-surface interactions. |
| Vascular Endothelial Growth Factor (VEGF) | Important signaling protein involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). | Angiogenesis: Enhances neo-vascularization in critical size bone defects. Also effective to induce bone regeneration in irradiated bone. |
| Von Willebrand Factor (vWF) | Its primary function is binding to other proteins, particularly Factor VIII. Also binds to collagen. It is important in platelet adhesion to wound sites. | Coagulation. |

shows the importance of EGF in wound healing in general. Therefore EGF may exert its positive activity in bone regeneration through its mitogenic characteristics.

### 2.5. Factor V, Factor XI, Factor XIII, Fibrinogen, Von Willebrand Factor (vWF)

Although Factor V, Factor XI, Factor XIII, Fibrinogen, and Von Willebrand Factor (vWF) play a central role in the coagulation cascade [13] (see also Table 1 for a brief description of their biological activity), an analysis of their roles in coagulation and wound repair goes beyond the scope of this review. Only one study reported the finding that Factor XIII supports bone healing [27]. Compared to the placebo injections at the site of the bone defect, in this animal study Factor XIII showed to favor the regeneration of bone with better biomechanical and histological qualities.

### 2.6. Basic Fibroblast Growth Factor (bFGF or FGF2)

FGFs comprise a growing family of structurally related polypeptide growth factors consisting of 22 members [18]. They transduce their signals through four high-affinity transmembrane protein-tyrosine kinases, called FGF receptors 1–4 (FGFR1–4). The role that basic Fibroblast Growth Factor (also known as FGF2 or bFGF) has in wound repair was shown by a study on FGF2 null mice [28]. These mice, when challenged by full-thickness excisional wounding showed delayed healing when compared to wild type mice. Another study also showed that the rate of wound re-epithelialization in FGF2 null mice is reduced and correlates well with a reduced collagen deposition at the wound site [29]. Other *in vitro* studies show the effect of FGF2 on bone-derived cells. For instance, one study shows that basic fibroblast growth factor (FGF2) induces proliferation and differentiation of rat bone marrow stromal cells [30]. More recently, the receptor-mediated binding, internalization, and processing of FGF2 during osteoblastic proliferative response was elucidated [31], and *in vivo* studies showed a potential role for FGF2 in bone regenerative therapy [32]. More specifically, a study [32] evaluated the effects of beta-tricalcium phosphate, collagen, and FGF2 on cortical bone repair of the tibial shaft in rabbits. When FGF2 was added to collagen and beta-tricalcium phosphate, the segmental bone defect was not only radiologically, but also mechanically healed with the presence of cortical bone 12 weeks after implantation. The role that FGF2 may have in induction of bone regeneration was also showed by a study that analyzed the healing of alveolar bone defects in beagle dogs. In this study, a histomorphometrical analysis showed that regenerated bone in FGF sites was significantly higher than in control (no FGF2) sites [33]. In terms of periodontal regeneration, a non-human primate study also showed that topical application of FGF2 can considerably enhance periodontal regeneration in surgically created furcation class II defects [34]. In conclusion, basic Fibroblast Growth Factor exerts its positive influence on bone regeneration though its proliferation and differentiation activity on preosteoblasts.

### 2.7. Fibronectin

Cell adhesion to extracellular matrices is essential to the development, maintenance, and remodeling of osseous tissues [35,36]. Fibronectin, being an extracellular matrix component, mediates adhesive interactions and plays a central role in osteoblast survival, proliferation, differentiation, and matrix mineralization, as well as in bone formation [37–39]. Because of the crucial role of extracellular matrix-mediated adhesion in osteoblast functions, many novel approaches to bone regenerative therapy have been designed to deliver fibronectin into the bone defects [36]. For instance, a study evaluated the osteogenic effect of a fibrin–fibronectin sealing system combined with β-tricalcium phosphate as a carrier for recombinant human bone morphogenetic proteins (rhBMP4) in the rat calvarial defect model [40]. This study showed that the fibronectin system has osteoconductive potential and may be employed as a carrier for BMPs. Other authors proposed the use of a short peptide containing the integrin recognition motif (RGD) for improving the osteoconductive activity of some biomaterials [41]. However, because the biological activity of short adhesive peptides is significantly lower than that of the complete protein [42], the RGD-based biomaterials are yet to be efficiently developed and need further improvement before being effectively used in bone regenerative therapy [41]. In summary, it can be concluded that Fibronectin exerts its positive influence on bone regeneration though its proliferation and differentiation activity on osteoblasts.

### 2.8. Insulin-like Growth Factor-I (IGF-I)

The insulin-like growth factors are a family of cell signaling molecules that regulates cell proliferation and differentiation. The IGF family includes 3 ligands (insulin, IGF-I, and IGF-II), 3 cell-surface receptors (the insulin, IGF-I, and IGF-II/mannose 6-phosphate receptors), and at least 6 high-affinity IGF-binding proteins (IGFBPs), which bind circulating IGFs and modulate their biological actions [43]. IGF-I is growth hormone dependent and possesses greater growth promoting activity than IGF-II [44]. IGF-I stimulates osteoblastic cells in culture to proliferate and to synthesize bone matrix proteins [45,46]. IGF-I also stimulates mRNA expression for alkaline phosphatase, osteopontin and osteocalcin in bone marrow stromal cells [47]. Circulating levels of IGF-I and bone mineral density decrease with increasing age, and administration of IGF-I increases bone turnover in patients with low bone mineral density [44]. A large number of animal studies suggest a prominent role of IGF-I on skeletal repair but their results have varied and have not been conclusive [48,49]. IGF-I was also shown to be potentially effective in periodontal tissue regeneration [50,51]. Although further studies may be needed prior to making a conclusion, IGF-I may exert its positive influence on bone regeneration through its proliferation and differentiation activity on osteoblasts.

### 2.9. Osteocalcin

Osteocalcin (also called bone Gla protein) is a low molecular weight vitamin K-dependent protein abundantly found in bone matrix [52,53]. Although previous studies report that osteocalcin was exclusively synthesized by osteoblasts and odontoblasts, other investigations showed that osteocalcin mRNA is not restricted to cells of mineralized tissues, but is also found in megakaryocytes and peripheral blood platelets [14]. The precise biological function of osteocalcin has not yet been fully elucidated. Some investigators have suggested that osteocalcin may act either as a cytokine or as a chemoattractant for osteoblasts, osteoclasts, and blood monocytes [52,54]. Elevations of serum osteocalcin were shown to correlate with high bone turnover states, including rapid skeletal growth [55,56] and skeletal fracture repair [57]. Lately, it has also been shown that osteocalcin is involved with endocrine regulation of sugar homeostasis by the skeleton [58]. Studies have also investigated the role of osteocalcin by a genetic approach [59] and have shown that osteocalcin-deficient mice show higher bone mass when compared to wild type littermates. This evidence supports the idea that osteocalcin is involved with regulation of bone formation; however, no studies have ever tested the beneficial or detrimental effects of osteocalcin in bone regenerative therapy.

### 2.10. P-Selectin (GMP-140)

Activated platelets, as well as stimulated endothelial cells, express P-selectin, a member of the selectin family of cell adhesion molecules [60]. P-selectin interacts with P-selectin glycoprotein ligand-1 (PSGL-1, CD162) during aggregation of activated platelets and for leukocyte rolling on stimulated endothelial cells. No studies were found that showed a direct effect of P-Selectin on preosteoblasts or on osteoblasts.

### 2.11. Platelet-derived Endothelial Cell Growth Factor (PDECGF or Thymidine phosphorylase)

PDECGF is a homodimer of 45 kDa subunits. It is a nucleoside metabolism enzyme associated with the maintenance of healthy mitochondria and the recovery of cells from pathological stress [61]. PDECGF was shown to have significant angiogenic activity *in vivo* [62]. No studies are available on the potential effects of PDECGF on preosteoblasts and on osteoblasts. Thus, so far there are only evidences that PDECGF may exert its positive effects on bone regeneration through its angiogenic activity.

### 2.12. Platelet-derived growth factor (PDGF)

PDGF from human platelets was purified and described as a cationic glycoprotein having a molecular weight of approximately 30 kDa and composed of two covalently linked subunits, designated as chains A (16–18 kDa) and B (14.5–16 kDa) [63]. In platelets, approximately 70% of the PDGF is present as AB dimers, with most of the remainder as BB [64]. PDGF elicits multifunctional actions on a variety of cells [65,66]. It is mitogenic to mesoderm-derived cells, such as fibroblasts, vascular smooth muscle cells, glial cells and chondrocytes. Also, it is a potent chemoattractant and activator of neutrophils, monocytes and fibroblasts. It increases the synthesis of phospholipids, cholesterol esters, glycogen and prostaglandins. Other actions of PDGF include its ability to regulate the synthesis and degradation of extracellular matrix proteins and to stimulate the synthesis of additional growth factors [66]. It is believed that PDGF plays an essential role in the cellular response to tissue injury, both as a stimulant of mesodermal cell growth and activity and as a chemoattractant to other cells involved in the repair process [66]. In this role PDGF appears to interact with Transforming Growth Factor-β1 (TGF-β1), which is also released by degranulating platelets at the source of the damaged tissue. *In vitro*, pulse application of platelet-derived growth factor on fetal rat osteoblastic cells enhances formation of a mineralizing matrix while continuous application is inhibitory [67]. Controlled release of PDGF is beneficial to bone regeneration when applied in bone defects [68] or in periodontal defects [69], whereas high doses of PDGF may inhibit the bone regenerative process [70,71]. It appears that the primary effect of PDGF in bone regeneration is related to its mitogenic activity [72,73].

### 2.13. Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is not only a neurotransmitter but also a hormone with various extraneuronal functions [74]. It is a potent mitogen and modulates the remodeling of various tissues such as liver and CNS [75–77]. Studies have also shown the presence of serotonin receptors in bone competent cells [78,79]. A recent study showed that serotonin was able to induce proliferation of osteoblast-like cells (MC3T3-E1) *in vitro*, and that serotonin-induced proliferation of MC3T3-E1 cells was also inhibited by the PKC inhibitor GF109203 and markedly reduced when antagonists of the serotonin receptors 5-HT2B/C or 5-HT2A/C were added [80]. Also, serotonin increased osteoprotegerin (OPG) and decreased receptor activator of NF-kappaB ligand (RANKL) secretion from osteoblasts, suggesting a role in osteoblast-induced inhibition of osteoclast differentiation [80]. In addition, in serotonin transporter null mice (5-HTT (−/−) mice), cancellous bone volume in the lumbar vertebrae is reduced, with a trend toward decreased trabecular thickness and trabecular number [81]. In conclusion, Serotonin may exert its positive influence on bone regeneration through its proliferative activity on osteoblasts.

### 2.14. Transforming growth factor beta-1 (TGF-β1)

TGF-β and its family members – the nodals, activins, bone morphogenetic proteins (BMPs), myostatin, anti-Muellerian hormone (AMH) and others – control cell division, differentiation, migration, adhesion, organization and programmed cell death [82]. TGF-β1, a member of the TGF-β family, is released by platelets

during the initial inflammatory phase of bone healing [49]. Its effects begin within 24 h after injury and persist for about 10 days [83]. When released by platelet degranulation or actively secreted by macrophages, TGF-β1 acts as a paracrine growth factor, affecting proliferation of fibroblasts, marrow stem cells and the preosteoblasts [49]. Moreover, each of these target cells has the ability to synthesize and secrete its own TGF-β proteins to act on adjacent cells in a paracrine fashion or act on itself as an autocrine growth factor [84,85]. TGF-β1 therefore represents a means for sustaining and amplifying the osteoblastic activity during the healing of bone [49] and may represent one of the most important factors released by the platelets at the site of the bone injury.

### 2.15. Thrombospondin-1

Thrombospondins (TSPs) are a small family of secreted, modular glycoproteins [86]. TSP1 and TSP2 each form 450 kDa homotrimers, and they display considerable sequence homology. They have been termed adhesion-modulating or matricellular components of the extracellular matrix (ECM). They influence adhesion, migration, proliferation, survival and differentiation of a variety of cell types [87]. TSP1, besides being released by platelets during coagulation, is also upregulated in healing wounds in skin, muscle, and the central nervous system through expression by macrophages or microglia, fibroblasts, and endothelial cells [87]. Moreover, TSP1 has a dominant role in the activation TGF-β1 [88]. This interaction is of significance in the homeostatic function of TSP1. TSP1 and TSP2 are both expressed by mesenchymal cells and chondrocytes in developing cartilage. They are both potent anti-angiogenic factors in abnormal angiogenesis [89]. Although there are no studies that show a direct effect of TSP1 on osteoblasts and on bone regeneration, the multiple activity of TSP1 in both angiogenesis and cell-surface interactions may have an influence on the regulation of bone regeneration.

### 2.16. Vascular Endothelial Growth Factor (VEGF)

The VEGF family currently includes five isoforms of VEGF (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E) and placenta growth factor (PLGF) [18]. The VEGF isoforms are generated as a result of alternative splicing from a single VEGF gene [90]. They exert their biological functions by binding to three different transmembrane tyrosine kinase receptors, designated VEGFR-1, VEGFR-2, and VEGFR-3 [18]. The biological functions of VEGF-A and its receptors VEGFR-1 and VEGFR-2 have been characterized in most detail. Based on a series of *in vitro* and *in vivo* studies, VEGF-A was identified as a major regulator of vasculogenesis and angiogenesis during development [91], indicating that it might also be involved in the regulation of angiogenesis during wound healing. Recent studies have also shown that the expression of VEGF is regulated by the hypoxia-inducible factor alpha (Hif1 alpha) as HIF alpha promotes angiogenesis and osteogenesis by elevating the levels of VEGF in osteoblasts [92]. Because of its angiogenic activity, VEGF has an important role in bone tissue regeneration. An *in vivo* study investigated the influence of the controlled release of recombinant human vascular endothelial growth factor (rhVEGF(165)) on angiogenesis and osteogenesis in a mandibular defect model, concluding that the activation of angiogenesis using rhVEGF(165) leads to more intensive angiogenesis and bone regeneration [93]. Another study showed that angiogenesis and osteogenesis can be promoted by the delivery of a gene encoding VEGF in atrophic nonunions in rabbits [94]. Very interestingly, VEGF delivered in irradiated rat calvaria osseous defects can enhance neo-vascularization and bone regeneration, outlining a novel approach for engineering tissues in hypovascular environments [95].

## 3. How platelet-rich plasma can be helpful in bone reconstruction therapy

By analyzing the effect that each of the biological factors released by the platelets can have on osteo-competent cells, it can be concluded that the rationale for using PRP in bone regenerative therapy dwells on the consideration that activated platelets, by releasing their growth and adhesive factors within a bone defect, may promote bone regeneration. The review of the literature on platelet-released factors is compelling and strongly supports the rationale for the use of platelets in bone regeneration. Therefore, it is surprising that the use of PRP for bone reconstruction therapy has produced many controversial results [9,10,96].

One of the key factors that may help understanding such controversial results is the method of preparation of PRP. As mentioned above, PRP is prepared *ex vivo* from autologous blood and is defined as a certain volume of plasma that has a platelet concentration several fold above the physiological levels. The standard protocol prepares PRP from autologous blood by a 2-step centrifugation process: the separation step and the concentration step [8,12,97]. For the separation step, aliquots of whole blood are collected in tubes containing acid-citrate–dextrose as an anti-coagulant. Immediately after being drawn, blood is centrifuged to separate red blood cells from platelets and plasma. For the concentration step, the supernatant composed of platelets and plasma is collected and centrifuged again in order to pellet the platelets. After the second centrifugation, the PRP is prepared by re-suspending the platelets in an appropriate volume of plasma to achieve the desired platelet concentration. Next, a combination of bovine thrombin and calcium chloride is used to activate the platelets and to obtain the "activated" PRP. The activated PRP appears as a gel due to the incorporation of the platelets into a web of fibrin [8,12].

By analyzing the existing literature and by comparing the reported methods of PRP preparation to the standard method reported above, 3 variables were identified that may be responsible for the differences among the results seen in the literature: (1) the concentration of the platelets in PRP; (2) the protocol for the activation of the platelets in PRP; (3) other variations from the standard protocol. Some biases that are independent from the PRP preparation protocol and that, nevertheless, may be responsible for the variability of the results were also found. They were named: (4) other biases of the studies.

### 3.1. The concentration of platelets in PRP

Clearly, the concentration of platelets in PRP may vary according to the procedure used for its preparation and more specifically according to the amount of plasma used to re-suspend the platelets [9]. The existing literature gives a clear indication of the most effective platelet concentration to be used in the PRP. Recent *in vitro* studies [98,99] tested the effects of platelet concentration on the proliferation and differentiation of primary osteoblasts and fibroblasts. Both these studies tend to suggest that low concentrations (few fold above the physiological levels) are more efficient than very high concentrations in inducing *in vitro* proliferation and differentiation. Another *in vivo* study [100] shows that the platelet concentration required for a positive PRP effect on bone regeneration spans a limited range (moderate concentrations, approximately 2- to 6-fold above the physiological level) and that lower concentrations have suboptimal effects, while higher concentrations might have a paradoxically inhibitory effect. These studies support the concept that the variability in the PRP's platelet concentration may indeed be responsible for the published variable results [101]. This is especially true when considering that various authors use different centrifuges and different g forces to spin the

blood samples, therefore obtaining PRPs that, even at equal volumes, may still differ in terms of platelet concentrations [9,102].

### 3.2. The activation of the platelets in PRP

In the literature, only one study effectively describes a dose response activation of platelets with thrombin [12]. This study clearly shows that human PRP, when activated by bovine thrombin and calcium chloride (142.8 U/ml of thrombin and 14.3 mg/ml of $CaCl_2$), releases the platelet's growth factors within a concentration range that is biologically active. Lower concentrations of thrombin and $CaCl_2$ or the use of thrombin alone and $CaCl_2$ alone do not allow for the complete release of the platelet's factors and also for the achievement of the same biological effects. Clearly, PRP needs to be correctly activated by an agonist in order to achieve full degranulation of the platelets' biological factors. Importantly, the concentrations of thrombin and $CaCl_2$ used in this *in vitro* study are equivalent to those proposed in the study by Marx et al. [8], one of the most convincing clinical studies on the beneficial effect of PRP in bone regeneration. Thus, it is not surprising that large and statistically sound studies such as those by Schlegel et al. [103], Wiltfang et al. [104], and Jakse et al. [105] that utilized an improper protocol for the activation of PRP (PRP was not activated by an agonist such as thrombin) do not show beneficial effects of PRP in bone regeneration. A recent study also showed that PRP does not enhance the healing of human periodontal intrabony defects when combined with beta-tricalcium phosphate for guided tissue regeneration [106]. However, in this study human PRP was activated with 100 U/ml of bovine thrombin as opposed to the 142.8 U/ml of bovine thrombin required for the maximum activation of the platelets [12]. Therefore, no conclusions on the efficacy of PRP in bone regenerative therapy can be drawn based on these studies.

### 3.3. Other variations from the standard protocol

A well-controlled and statistically significant study [107] did not find an effect of PRP in the enhancement of titanium osseointegration in dogs. In this case, however, PRP was prepared upon collection of whole blood in vials containing EDTA rather than citrate-based anti-coagulants (as per the standard protocol [8,12]). Moreover, in this study the histomorphometry analysis was performed only at three weeks, a time point that may prove too early to detect differences in bone formation. A recent study by Roldan et al. [108] conducted on 28 rats compared the osteoinductive activity of PRP and rhBMP7 when combined with either autologous bone or inorganic bovine bone in non-critical size bone defects. Surprisingly, in this study the histomorphometry analysis showed that neither rhBMP7 nor PRP were able to accelerate bone growth when combined with autologous bone grafts. A stimulating effect was only observed when rhBMP7 was combined with anorganic bovine bone. PRP did not show any beneficial effect when combined with anorganic bovine bone. It has to be considered, however, that in this study the rat PRP was activated with human thrombin. Human thrombin may not be optimal in inducing the rat platelets' activation and degranulation. Indeed, studies have shown large interspecies variations when PRP is prepared and activated with thrombin [109]. The same bias may contribute to the differential effects of PRP when combined with hydroxyapatite for sinus grafting in minipigs [110]. This study shares the negative outcomes with another study [107] performed on dogs where bovine thrombin was used for activation of dog autologous PRP. Another randomized and well-controlled study evaluated the regenerative influence of PRP added to xenogenic bone grafts on bone histomorphometric parameters in a dog model [111]. This study found that the addition of PRP to xenogenic bone grafts demonstrated a low regenerative potential in this animal model. Again, in this case, the canine PRP was activated by bovine thrombin.

Therefore, the variability of the results seen in many studies may be due to the various activation protocols utilized, including the use of alternatives to thrombin and the use of various allogenic thrombins in different animal models.

### 3.4. Other biases of the studies

A study by Choi et al. [112] suggests that the addition of PRP in autogenous bone graft retards new bone formation when tested in mandibular critical size bone defects in dogs. In this study, the bone formation was evaluated using the sequential administration of fluorescent dyes followed by histological fluorescent and morphometrical evaluations. Although the authors claim that an evaluation of bone formation at 6 weeks may be representative of newly formed bone, other studies suggest that bone maturation in mandibular critical size defects in dogs should be evaluated at later time points, with the presence of cortical bone demonstrated four to six months after surgery [113,114]. In other cases, it can be observed that an inappropriate statistical power of the studies can be accounted for the insignificant results. For instance, the study by Froum et al. [115] claims histomorphometrical differences in bone regeneration among samples of only three surgical cases. Shanaman et al. [116] claim the non-beneficial effect of PRP in procedures of bone regeneration based on a non-controlled study, where the conclusion is inferred simply by comparing the results of the surgeries performed using DFDBA and PRP to the results of studies previously published by others. A study by Arpornmaeklong et al. [117] shows a dose dependent stimulation of rat bone marrow cell proliferation by the rat PRP activated with bovine thrombin. In this study PRP also reduces the Alkaline Phosphatase Activity (ALP) and the calcium content in the supernatant of these cell cultures. This study, by comparing PRP to rhBMP2 *in vitro*, concludes that PRP is not a substitute for BMP2 in osteogenic induction. This conclusion, however, is based on the evaluation of the sole ALP and is also biased by an unsupported protocol for the activation of PRP.

On the other hand, some literature supports the use of PRP but still presents variations from the standard protocols. For instance, an *in vitro* study [118] shows that PRP acts as a mitogen on osteoblastic cells and PDL cells but acts as a growth inhibitor on epithelial cells. However, in this study PRP was not activated by an agonist. Another study testing human PRP [119], again not activated by an agonist, demonstrates that PRP is also able to upregulate the collagen synthesis of osteoblast and PDL cell cultures. Other studies report beneficial effects of PRP in both periodontal and cosmetic surgeries [120–122]. However, being case reports, these studies are not controlled and therefore should not be considered as testimonials of the beneficial activity of PRP in regenerative surgery. An *in vivo* randomized, blind study that evaluated the formation of new bone by means of histomorphometry shows increased bone formation with the addition of PRP in rabbits [123]. However, in this study the rabbit PRP was activated by bovine thrombin and because no study has ever shown full activation of rabbit platelets by bovine thrombin, the result should not be considered in support of the use of PRP in bone regenerative therapy. Another recent study examined the effect of the use of PRP in combination with autologous bone grafts in a rabbit segmental radial defect model [124]. Again, in this study the rabbit's PRP was activated by bovine thrombin. The study showed that PRP has stimulatory effects on bone formation as measured by histomorphometrical analysis. In two other *in vivo* studies [125,126], goat PRP was prepared and activated with bovine thrombin and calcium chloride using the concentration suggested in other studies [8,12]. These studies show that bone healing was considerably enhanced by the PRP despite the use of bovine thrombin for

activation of goat PRP. Yet, being well designed and well controlled in terms of statistical power, both these studies may be quite significant and supportive for the use of PRP. Indeed, in these studies 28 goats underwent a continuity resection of the mandibular angle followed by a primary reconstruction using autologous bone with or without the addition of PRP. Both the radiographic and histomorphometric evaluations show a statistically significant difference in bone formation between the two groups, with more bone and no fibrous encapsulation of the scaffold in cases associated with the use of PRP. Interestingly, in these two studies the histomorphometry also evaluates the capillary formation assessed by the presence of endothelial cells and erythrocytes. At six and twelve weeks post implantation, the group treated with PRP always showed higher counts of capillaries, an extremely important feature that may favor the bone formation in critical size defects, where blood supply is always compromised. Another randomized human clinical study evaluated the efficacy of PRP combined with hydroxyapatite for treatment of interproximal intrabony osseous defects in 70 subjects diagnosed with chronic periodontitis [127]. In this study the PRP was not activated with thrombin and was rather mixed with sodium alginate to achieve a gel able to be easily combined with the hydroxyapatite. Thirty-five subjects were randomly assigned to either the test group (PRP and HA) or the control group (HA with saline). The treatment with the PRP and HA led to a significantly more favorable clinical improvement.

### 3.5. Clinical studies

The first clinical study using PRP for bone reconstruction therapy was performed by Marx et al. [8]. In this randomized study, 88 patients with mandibular defects were treated with autogenous cancellous bone grafts with or without the addition of activated PRP. Both the radiographic and the histomorphometric evaluation show a significantly greater percentage of bone with the addition of PRP. Another randomized, split mouth, double-masked clinical trial [128] compared the clinical outcomes obtained by the combination of activated PRP and a bovine derived xenograft (BDX) to those obtained from the use of BDX alone in the treatment of periodontal intrabony defects. In this study, the addition of PRP significantly improved their clinical outcomes. Another study tested the transplantation of culture expanded bone marrow cells (BMC) in distraction osteogenesis of the long bones with or without the addition of activated PRP [129]. The BMC-PRP group consisted of 32 bone defects (14 femora, 18 tibiae) in 17 patients while the BMC group consisted of 60 bones (25 femora, 35 tibiae) in 29 patients. This study concluded that transplantation of BMC in association to PRP shortened the treatment period and reduced the associated complications by accelerating new bone formation. The effect of PRP in the treatment of periodontal intrabony defects in humans has also been investigated [130]. Seventeen intrabony defects were randomly treated with either activated PRP and bovine porous bone mineral (BPBM) (test group, $n = 9$) or with BPBM alone (control group, $n = 8$). The treatment with the combination of PRP and BPBM led to significantly favorable clinical improvements in periodontal intrabony defects compared to the use of BPBM alone. Recently, a randomized, double-masked clinical trial compared the use of the combination of demineralized bone (DFDBA) and PRP to the use of demineralized bone alone in treatment of human periodontal intrabony defects [131]. This study shows the efficacy of the use of thrombin-activated PRP in combination with DFDBA as both the probing depth reduction and the clinical attachment gain were statistically greater in the test group as opposed to the group treated with DFDBA alone.

All these clinical studies present one thing in common: the use of human PRP prepared and activated by bovine thrombin and calcium chloride following the only protocol proven to maximize the platelet activation [8,12]. The platelet's concentration – moderate concentrations, approximately 4- to 8-fold – and the level of activation – highest with high concentrations of bovine thrombin and calcium chloride (142.8 U/ml of thrombin and 14.3 mg/ml of $CaCl_2$) – are also the same for all them.

## 4. Guidelines for the use of platelet-rich plasma in bone reconstruction therapy

Based on the analysis of the above mentioned studies, it can be gathered that when PRP is used following a specific preparation and a specific activation protocol [8,12] it can be beneficial to the bone regenerative therapy. It should be further noted that in the clinical studies mentioned above [8,128,130,131] all but one [129] delivered PRP within the bony defect in combination with a bone filler such as demineralized allografts, xenografts, or autologous bone. Growth factors have a very short half-life and an appropriate delivery system is required to deliver them in a time-controlled fashion in order to achieve a therapeutic effect on the target cells [132]. Thus, it may be possible that the bone fillers have worked as a growth factor delivery system. The study that did not deliver PRP in combination with a bone filler did so in combination with bone marrow-derived stromal cells. These cells may have been stimulated by PRP factors at the time of cell transplantation into the bone defect, therefore avoiding the need for a controlled release of the PRP factors.

Certain clinical situations may need to be taken into consideration when a PRP-based bone reconstruction therapy is attempted, such as the variability of platelet concentration among patients. Prior to surgery, a platelet count should be requested in order to adjust the amount of plasma used to re-suspend the platelets during the concentration step. The biological environment into which the PRP is delivered may also influence the outcome of the PRP-based therapy. Large defects may show lower levels of bone regeneration as compared to smaller defects. This is true for all the existing regeneration therapies and is due to the limited amount of mesenchymal cells present in larger defects.

The current studies suggest that a dental or orthopaedic surgeon who plans to use PRP for bone reconstruction therapy should carefully plan to prepare PRP with a platelet concentration 4- to 8-fold above the average physiological levels. He or she should also plan to activate the PRP with 142.8 U/ml of bovine thrombin and 14.3 mg/ml of $CaCl_2$ in order to maximize the release of the biological factors from the platelets' alpha and dense granules. A bone filler that may work as carrier for the biologically active factors released by the platelet should also be utilized in combination with PRP.

Surgeons are hesitant to use a bovine derivative in human bone regenerative procedures as numerous reports have documented the development of anti-bovine antibodies that cross reacted with human clotting factors in response to the use of bovine products to provide hemostasis. In some well-described cases, these antibodies have led to clinical syndromes that range from severe postoperative bleeding to high rates of thrombosis [133–141]. Therefore, the substitution of thrombin is needed in order to effectively implement PRP in bone reconstruction therapy. It has been shown that the use of a combination of calcium sulfate and PRP (CS-Platelet) represents a valid alternative to the activation of PRP by thrombin. Studies have shown that in this combination calcium sulfate acts as an activator of the platelets [142] as well as a delivery system for the platelet-released growth factors [143]. Animal and human studies with CS-Platelet used for bone regeneration have shown that CS-Platelet is able to induce formation of bone in heterotopic and orthotopic sites, and in orthotopic critical size bone defects [144]. Based on these experimental and clinical results, CS-Platelet

may represent a cost-effective treatment in bone reconstruction therapy and an adjuvant to the current treatments. For instance, one may envision a novel combinatorial approach with CS-Platelet and rhBMP2 or rhBMP7, in which the osteoinductive activity of CS-Platelet based on the proliferative activity of PRP and on the osteoconductive activity of calcium sulfate is further supported by the potent osteodifferentiation and osteoinduction induced by the BMPs. This combinatorial approach, by imitating the proliferation and differentiation phases of bone wound healing, may represent the most successful approach in bone regenerative therapy. Future studies are needed to evaluate these combinatorial approaches.

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## References

[1] Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, et al. Recombinant human bone morphogenetic protein induces bone formation. Proc Natl Acad Sci U S A 1990;87(6):2220–4.
[2] Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation: molecular clones and activities. Science 1988;242(4885):1528–34.
[3] Govender S, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, et al. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am 2002;84-A(12):2123–34.
[4] Szpalski M, Gunzburg R. Recombinant human bone morphogenetic protein-2: a novel osteoinductive alternative to autogenous bone graft? Acta Orthop Belg 2005;71(2):133–48.
[5] Termaat MF, Den Boer FC, Bakker FC, Patka P, Haarman HJ. Bone morphogenetic proteins. Development and clinical efficacy in the treatment of fractures and bone defects. J Bone Joint Surg Am 2005;87(6):1367–78.
[6] Kumar V, Cotran RS, Robbins SL. Tissue repair: cell regeneration and fibrosis. Basic pathology. 7th ed. Philadelphia: Saunders; 2003. p. 61–78.
[7] Gerstenfeld LC, Cullinane DM, Barnes GL, Graves DT, Einhorn TA. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem 2003;88(5):873–84.
[8] Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(6):638–46.
[9] Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62(4):489–96.
[10] Freymiller EG, Aghaloo TL. Platelet-rich plasma: ready or not? J Oral Maxillofac Surg 2004;62(4):484–8.
[11] Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res 2005;84(5):434–9.
[12] Lacoste E, Martineau I, Gagnon G. Platelet concentrates: effects of calcium and thrombin on endothelial cell proliferation and growth factor release. J Periodontol 2003;74(10):1498–507.
[13] Hoffman R, Benz EJJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, et al. Hematology: basic principles and practice. Philadelphia: Churchill Livingstone; 2000.
[14] Thiede MA, Smock SL, Petersen DN, Grasser WA, Thompson DD, Nishimoto SK. Presence of messenger ribonucleic acid encoding osteocalcin, a marker of bone turnover, in bone marrow megakaryocytes and peripheral blood platelets. Endocrinology 1994;135(3):929–37.
[15] Hoebertz A, Arnett TR, Burnstock G. Regulation of bone resorption and formation by purines and pyrimidines. Trends Pharmacol Sci 2003;24(6):290–7.
[16] Morrison MS, Turin L, King BF, Burnstock G, Arnett TR. ATP is a potent stimulator of the activation and formation of rodent osteoclasts. J Physiol 1998;511(Pt 2):495–500.
[17] Hoebertz A, Meghji S, Burnstock G, Arnett TR. Extracellular ADP is a powerful osteolytic agent: evidence for signaling through the P2Y(1) receptor on bone cells. FASEB J 2001;15(7):1139–48.
[18] Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003;83(3):835–70.
[19] Kampfer H, Pfeilschifter J, Frank S. Expressional regulation of angiopoietin-1 and -2 and the tie-1 and -2 receptor tyrosine kinases during cutaneous wound healing: a comparative study of normal and impaired repair. Lab Invest 2001;81(3):361–73.
[20] Castor CW, Miller JW, Walz DA. Structural and biological characteristics of connective tissue activating peptide (CTAP-III), a major human platelet-derived growth factor. Proc Natl Acad Sci U S A 1983;80(3):765–9.
[21] Deutsch V, Bitan M, Friedmann Y, Eldor A, Vlodavsky I. Megakaryocyte maturation is associated with expression of the CXC chemokine connective tissue-activating peptide CTAP III. Br J Haematol 2000;111(4):1180–9.
[22] Han ZC, Lu M, Li J, Defard M, Boval B, Schlegel N, et al. Platelet factor 4 and other CXC chemokines support the survival of normal hematopoietic cells and reduce the chemosensitivity of cells to cytotoxic agents. Blood 1997;89(7):2328–35.
[23] Steed DL. Modifying the wound healing response with exogenous growth factors. Clin Plast Surg 1998;25(3):397–405.
[24] Grayson LS, Hansbrough JF, Zapata-Sirvent RL, Dore CA, Morgan JL, Nicolson MA. Quantitation of cytokine levels in skin graft donor site wound fluid. Burns 1993;19(5):401–5.
[25] Marie PJ, Hott M, Perheentupa J. Effects of epidermal growth factor on bone formation and resorption in vivo. Am J Physiol 1990;258(2 Pt 1):E275–81.
[26] Wang K, Yamamoto H, Chin JR, Werb Z, Vu TH. Epidermal growth factor receptor-deficient mice have delayed primary endochondral ossification because of defective osteoclast recruitment. J Biol Chem 2004;279(51):53848–56.
[27] Claes L, Burri C, Gerngross H, Mutschler W. Bone healing stimulated by plasma factor XIII. Osteotomy experiments in sheep. Acta Orthop Scand 1985;56(1):57–62.
[28] Ortega S, Ittmann M, Tsang SH, Ehrlich M, Basilico C. Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. Proc Natl Acad Sci U S A 1998;95(10):5672–7.
[29] Miller DL, Ortega S, Bashayan O, Basch R, Basilico C. Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in FGF2 null mice. Mol Cell Biol 2000;20(6):2260–8.
[30] Fujita M, Kinoshita Y, Sato E, Maeda H, Ozono S, Negishi H, et al. Proliferation and differentiation of rat bone marrow stromal cells on poly(glycolic acid)–collagen sponge. Tissue Eng 2005;11(9–10):1346–55.
[31] Dupree MA, Pollack SR, Levine EM, Laurencin CT. Fibroblast growth factor 2 induced proliferation in osteoblasts and bone marrow stromal cells: a whole cell model. Biophys J 2006;91(8):3097–112.
[32] Komaki H, Tanaka T, Chazono M, Kikuchi T. Repair of segmental bone defects in rabbit tibiae using a complex of beta-tricalcium phosphate, type I collagen, and fibroblast growth factor-2. Biomaterials 2006;27(29):5118–26.
[33] Hosokawa R, Kikuzaki K, Kimoto T, Matsuura T, Chiba D, Wadamoto M, et al. Controlled local application of basic fibroblast growth factor (FGF-2) accelerates the healing of GBR. An experimental study in beagle dogs. Clin Oral Implants Res 2000;11(4):345–53.
[34] Takayama S, Murakami S, Shimabukuro Y, Kitamura M, Okada H. Periodontal regeneration by FGF-2 (bFGF) in primate models. J Dent Res 2001;80(12):2075–9.
[35] Damsky CH. Extracellular matrix–integrin interactions in osteoblast function and tissue remodeling. Bone 1999;25(1):95–6.
[36] Garcia AJ, Reyes CD. Bio-adhesive surfaces to promote osteoblast differentiation and bone formation. J Dent Res 2005;84(5):407–13.
[37] Weiss RE, Reddi AH. Role of fibronectin in collagenous matrix-induced mesenchymal cell proliferation and differentiation in vivo. Exp Cell Res 1981;133(2):247–54.
[38] Moursi AM, Damsky CH, Lull J, Zimmerman D, Doty SB, Aota S, et al. Fibronectin regulates calvarial osteoblast differentiation. J Cell Sci 1996;109(Pt 6):1369–80.
[39] Zimmerman D, Jin F, Leboy P, Hardy S, Damsky C. Impaired bone formation in transgenic mice resulting from altered integrin function in osteoblasts. Dev Biol 2000;220(1):2–15.
[40] Han DK, Kim CS, Jung UW, Chai JK, Choi SH, Kim CK, et al. Effect of a fibrin–fibronectin sealing system as a carrier for recombinant human bone morphogenetic protein-4 on bone formation in rat calvarial defects. J Periodontol 2005;76(12):2216–22.
[41] Schaffner P, Dard MM. Structure and function of RGD peptides involved in bone biology. Cell Mol Life Sci 2003;60(1):119–32.
[42] Pierschbacher M, Hayman EG, Ruoslahti E. Synthetic peptide with cell attachment activity of fibronectin. Proc Natl Acad Sci U S A 1983;80(5):1224–7.
[43] Werner H, Katz J. The emerging role of the insulin-like growth factors in oral biology. J Dent Res 2004;83(11):832–6.
[44] Trippel SB. Potential role of insulinlike growth factors in fracture healing. Clin Orthop Related Res 1998;(355 Suppl.):S301–13.
[45] Conover CA. In vitro studies of insulin-like growth factor I and bone. Growth Horm IGF Res 2000;10(Suppl. B):S107–10.
[46] Canalis E. Effect of insulinlike growth factor I on DNA and protein synthesis in cultured rat calvaria. J Clin Invest 1980;66(4):709–19.
[47] Tanaka H, Quarto R, Williams S, Barnes J, Liang CT. In vivo and in vitro effects of insulin-like growth factor-I (IGF-I) on femoral mRNA expression in old rats. Bone 1994;15(6):647–53.
[48] Barnes GL, Kostenuik PJ, Gerstenfeld LC, Einhorn TA. Growth factor regulation of fracture repair. J Bone Miner Res 1999;14(11):1805–15.
[49] Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury 2005;36(12):1392–404.

[50] Chen FM, Zhao YM, Wu H, Deng ZH, Wang QT, Zhou W, et al. Enhancement of periodontal tissue regeneration by locally controlled delivery of insulin-like growth factor-I from dextran-co-gelatin microspheres. J Control Release 2006;114(2):209–22.
[51] Giannobile WV, Finkelman RD, Lynch SE. Comparison of canine and non-human primate animal models for periodontal regenerative therapy: results following a single administration of PDGF/IGF-I. J Periodontol 1994; 65(12):1158–68.
[52] Price PA. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. Vitam Horm 1985;42:65–108.
[53] Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev 1989;69(3):990–1047.
[54] Malone JD, Teitelbaum SL, Griffin GL, Senior RM, Kahn AJ. Recruitment of osteoclast precursors by purified bone matrix constituents. J Cell Biol 1982;92(1):227–30.
[55] Epstein S, Poser J, McClintock R, Johnston Jr CC, Bryce G, Hui S. Differences in serum bone GLA protein with age and sex. Lancet 1984;1(8372):307–10.
[56] Johansen JS, Giwercman A, Hartwell D, Nielsen CT, Price PA, Christiansen C, et al. Serum bone Gla-protein as a marker of bone growth in children and adolescents: correlation with age, height, serum insulin-like growth factor I, and serum testosterone. J Clin Endocrinol Metab 1988;67(2):273–8.
[57] Slovik DM, Gundberg CM, Neer RM, Lian JB. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. J Clin Endocrinol Metab 1984;59(2):228–30.
[58] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130(3):456–69.
[59] Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, et al. Increased bone formation in osteocalcin-deficient mice. Nature 1996;382(6590):448–52.
[60] Chen M, Geng JG. P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis. Arch Immunol Ther Exp 2006;54(2):75–84.
[61] Toi M, Atiqur Rahman M, Bando H, Chow LW. Thymidine phosphorylase (platelet-derived endothelial-cell growth factor) in cancer biology and treatment. Lancet Oncol 2005;6(3):158–66.
[62] Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 1989;338(6216):557–62.
[63] Raines EW, Ross R. Purification of human platelet-derived growth factor. Methods Enzymol 1985;109:749–73.
[64] Ross R. Platelet-derived growth factor. Lancet 1989;1(8648):1179–82.
[65] Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. Cell 1986;46(2):155–69.
[66] Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc 2006;81(9):1241–57.
[67] Hsieh SC, Graves DT. Pulse application of platelet-derived growth factor enhances formation of a mineralizing matrix while continuous application is inhibitory. J Cell Biochem 1998;69(2):169–80.
[68] Park YJ, Lee YM, Lee JY, Seol YJ, Chung CP, Lee SJ. Controlled release of platelet-derived growth factor-BB from chondroitin sulfate–chitosan sponge for guided bone regeneration. J Control Release 2000;67(2–3):385–94.
[69] Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor-modulated guided tissue regenerative therapy. J Periodontol 1995;66(6):522–30.
[70] Marden LJ, Fan RS, Pierce GF, Reddi AH, Hollinger JO. Platelet-derived growth factor inhibits bone regeneration induced by osteogenin, a bone morphogenetic protein, in rat craniotomy defects. J Clin Invest 1993;92(6):2897–905.
[71] Nevins M, Giannobile WV, McGuire MK, Kao RT, Mellonig JT, Hinrichs JE, et al. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. J Periodontol 2005;76(12):2205–15.
[72] Canalis E, McCarthy TL, Centrella M. Effects of platelet-derived growth factor on bone formation in vitro. J Cell Physiol 1989;140(3):530–7.
[73] Hughes FJ, Aubin JE, Heersche JN. Differential chemotactic responses of different populations of fetal rat calvaria cells to platelet-derived growth factor and transforming growth factor beta. Bone Miner 1992;19(1):63–74.
[74] Veenstra-VanderWeele J, Anderson GM, Cook Jr EH. Pharmacogenetics and the serotonin system: initial studies and future directions. Eur J Pharmacol 2000;410(2–3):165–81.
[75] Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. Science 2006;312(5770): 104–7.
[76] Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, et al. Serotonin regulates mammary gland development via an autocrine–paracrine loop. Dev Cell 2004;6(2):193–203.
[77] Vitalis T, Parnavelas JG. The role of serotonin in early cortical development. Dev Neurosci 2003;25(2–4):245–56.
[78] Westbroek I, van der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ. Expression of serotonin receptors in bone. J Biol Chem 2001;276(31):28961–8.
[79] Bliziotes MM, Eshleman AJ, Zhang XW, Wiren KM. Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. Bone 2001;29(5):477–86.
[80] Gustafsson BI, Thommesen L, Stunes AK, Tommeras K, Westbroek I, Waldum HL, et al. Serotonin and fluoxetine modulate bone cell function in vitro. J Cell Biochem 2006;98(1):139–51.
[81] Bliziotes M, Gunness M, Eshleman A, Wiren K. The role of dopamine and serotonin in regulating bone mass and strength: studies on dopamine and serotonin transporter null mice. J Musculoskelet Neuronal Interact 2002;2(3):291–5.
[82] Massague J, Gomis RR. The logic of TGFbeta signaling. FEBS Lett 2006;580(12):2811–20.
[83] Hollinger J, Wong ME. The integrated processes of hard tissue regeneration with special emphasis on fracture healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82(6):594–606.
[84] Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994;8(2):133–46.
[85] Massague J. The transforming growth factor-beta family. Annu Rev Cell Biol 1990;6:597–641.
[86] Alford AI, Hankenson KD. Matricellular proteins: extracellular modulators of bone development, remodeling, and regeneration. Bone 2006;38(6):749–57.
[87] Adams JC. Thrombospondins: multifunctional regulators of cell interactions. Annu Rev Cell Dev Biol 2001;17:25–51.
[88] Schultz-Cherry S, Chen H, Mosher DF, Misenheimer TM, Krutzsch HC, Roberts DD, et al. Regulation of transforming growth factor-beta activation by discrete sequences of thrombospondin 1. J Biol Chem 1995;270(13):7304–10.
[89] Armstrong LC, Bornstein P. Thrombospondins 1 and 2 function as inhibitors of angiogenesis. Matrix Biol 2003;22(1):63–71.
[90] Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999;13(1):9–22.
[91] Gale NW, Yancopoulos GD. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. Genes Dev 1999;13(9):1055–66.
[92] Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J Clin Invest 2007;117(6):1616–26.
[93] Kleinheinz J, Stratmann U, Joos U, Wiesmann HP. VEGF-activated angiogenesis during bone regeneration. J Oral Maxillofac Surg 2005;63(9): 1310–6.
[94] Geiger F, Bertram H, Berger I, Lorenz H, Wall O, Eckhardt C, et al. Vascular endothelial growth factor gene-activated matrix (VEGF165-GAM) enhances osteogenesis and angiogenesis in large segmental bone defects. J Bone Miner Res 2005;20(11):2028–35.
[95] Kaigler D, Wang Z, Horger K, Mooney DJ, Krebsbach PH. VEGF scaffolds enhance angiogenesis and bone regeneration in irradiated osseous defects. J Bone Miner Res 2006;21(5):735–44.
[96] Schmitz JP, Hollinger JO. The biology of platelet-rich plasma. J Oral Maxillofac Surg 2001;59(9):1119–21.
[97] Dwyer SD, Meyers KM. Anesthetics and anticoagulants used in the preparation of rat platelet-rich-plasma alter rat platelet aggregation. Thromb Res 1986;42(2):139–51.
[98] Choi BH, Zhu SJ, Kim BY, Huh JY, Lee SH, Jung JH. 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(4):420–4.
[99] Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res 2006;17(2):212–9.
[100] 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(4):665–71.
[101] Pietrzak WS, Eppley BL. Platelet rich plasma: biology and new technology. J Craniofac Surg 2005;16(6):1043–54.
[102] Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 2001;10(4):225–8.
[103] Schlegel KA, Donath K, Rupprecht S, Falk S, Zimmermann R, Felszeghy E, et al. De novo bone formation using bovine collagen and platelet-rich plasma. Biomaterials 2004;25(23):5387–93.
[104] Wiltfang J, Kloss FR, Kessler P, Nkenke E, Schultze-Mosgau S, Zimmermann R, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical-size defects. An animal experiment. Clin Oral Implants Res 2004;15(2):187–93.
[105] Jakse N, Tangl S, Gilli R, Berghold A, Lorenzoni M, Eskici A, et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res 2003;14(5):578–83.
[106] Dori F, Huszar T, Nikolidakis D, Tihanyi D, Horvath A, Arweiler NB, et al. Effect of platelet-rich plasma on the healing of intrabony defects treated with beta tricalcium phosphate and expanded polytetrafluoroethylene membranes. J Periodontol 2008;79(4):660–9.
[107] Jensen TB, Rahbek O, Overgaard S, Soballe K. Platelet rich plasma and fresh frozen bone allograft as enhancement of implant fixation. An experimental study in dogs. J Orthop Res 2004;22(3):653–8.
[108] Roldan JC, Jepsen S, Miller J, Freitag S, Rueger DC, Acil Y, et al. Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. Bone 2004;34(1):80–90.
[109] Dolder JV, 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.
[110] Furst G, Gruber R, Tangl S, Zechner W, Haas R, Mailath G, et al. Sinus grafting with autogenous platelet-rich plasma and bovine hydroxyapatite. A histomorphometric study in minipigs. Clin Oral Implants Res 2003;14(4): 500–8.

[111] Sanchez AR, Sheridan PJ, Eckert SE, Weaver AL. Regenerative potential of platelet-rich plasma added to xenogenic bone grafts in peri-implant defects: a histomorphometric analysis in dogs. J Periodontol 2005;76(10):1637–44.
[112] Choi BH, Im CJ, Huh JY, Suh JJ, Lee SH. Effect of platelet-rich plasma on bone regeneration in autogenous bone graft. Int J Oral Maxillofac Surg 2004;33(1):56–9.
[113] Cummings CW. Experimental observations of canine mandibular regeneration following segmental removal, freezing, and reimplantation. Ann Otol Rhinol Laryngol Suppl 1978;87(6 Pt 3 Suppl. 54):1–11.
[114] Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop 1986;(205):299–308.
[115] Froum SJ, Wallace SS, Tarnow DP, Cho SC. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. Int J Periodontics Restorative Dent 2002;22(1):45–53.
[116] Shanaman R, Filstein MR, Danesh-Meyer MJ. Localized ridge augmentation using GBR and platelet-rich plasma: case reports. Int J Periodontics Restorative Dent 2001;21(4):345–55.
[117] Arpornmaeklong P, Kochel M, Depprich R, Kubler NR, Wurzler KK. Influence of platelet-rich plasma (PRP) on osteogenic differentiation of rat bone marrow stromal cells. An in vitro study. Int J Oral Maxillofac Surg 2004;33(1):60–70.
[118] Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, et al. Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally related cells in vitro. J Periodontol 2003;74(6):849–57.
[119] Kawase T, Okuda K, Wolff LF, Yoshie H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol 2003;74(6):858–64.
[120] 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 Reconstr Surg 2001;107(1):229–37. Discussion 238–229.
[121] Mazor Z, Peleg M, Garg AK, Luboshitz J. Platelet-rich plasma for bone graft enhancement in sinus floor augmentation with simultaneous implant placement: patient series study. Implant Dent 2004;13(1):65–72.
[122] Kassolis JD, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol 2000;71(10):1654–61.
[123] Aghaloo TL, Moy PK, Freymiller EG. Evaluation of platelet-rich plasma in combination with anorganic bovine bone in the rabbit cranium: a pilot study. Int J Oral Maxillofac Implants 2004;19(1):59–65.
[124] Kroese-Deutman HC, Vehof JW, Spauwen PH, Stoelinga PJ, Jansen JA. Orthotopic bone formation in titanium fiber mesh loaded with platelet-rich plasma and placed in segmental defects. Int J Oral Maxillofac Surg 2008;37(6):542–9.
[125] Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstruction: a clinical and radiographic animal study on the use of autogenous scaffolds and platelet-rich plasma. Int J Oral Maxillofac Surg 2002;31(3):281–6.
[126] Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstruction: a histological and histomorphometric study on the use of autogenous scaffolds, particulate cortico-cancellous bone grafts and platelet rich plasma in goats. Int J Oral Maxillofac Surg 2004;33(1):48–55.
[127] Okuda K, Tai H, Tanabe K, Suzuki H, Sato T, Kawase T, 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(6):890–8.
[128] Hanna R, Trejo PM, Weltman RL. Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. J Periodontol 2004;75(12):1668–77.
[129] Kitoh H, Kitakoji T, Tsuchiya H, Katoh M, Ishiguro N. Transplantation of culture expanded bone marrow cells and platelet rich plasma in distraction osteogenesis of the long bones. Bone 2006.
[130] Ouyang XY, Qiao J. Effect of platelet-rich plasma in the treatment of periodontal intrabony defects in humans. Chin Med J 2006;119(18):1511–21.
[131] Piemontese M, Aspriello SD, Rubini C, Ferrante L, Procaccini M. Treatment of periodontal intrabony defects with demineralized freeze-dried bone allograft in combination with platelet-rich plasma: a comparative clinical trial. J Periodontol 2008;79(5):802–10.
[132] Nimni ME. Polypeptide growth factors: targeted delivery systems. Biomaterials 1997;18(18):1201–25.
[133] Sands JJ, Nudo SA, Ashford RG, Moore KD, Ortel TL. Antibodies to topical bovine thrombin correlate with access thrombosis. Am J Kidney Dis 2000;35(5):796–801.
[134] Chouhan VD, De La Cadena RA, Nagaswami C, Weisel JW, Kajani M, Rao AK. Simultaneous occurrence of human antibodies directed against fibrinogen, thrombin, and factor V following exposure to bovine thrombin: effects on blood coagulation, protein C activation and platelet function. Thromb Haemost 1997;77(2):343–9.
[135] Christie RJ, Carrington L, Alving B. Postoperative bleeding induced by topical bovine thrombin: report of two cases. Surgery 1997;121(6):708–10.
[136] Dorion RP, Hamati HF, Landis B, Frey C, Heydt D, Carey D. Risk and clinical significance of developing antibodies induced by topical thrombin preparations. Arch Pathol Lab Med 1998;122(10):887–94.
[137] Israels SJ, Israels ED. Development of antibodies to bovine and human factor V in two children after exposure to topical bovine thrombin. Am J Pediatr Hematol Oncol 1994;16(3):249–54.
[138] Lawson JH. The clinical use and immunologic impact of thrombin in surgery. Semin Thromb Hemost 2006;32(Suppl. 1):98–110.
[139] Sarfati MR, Dilorenzo DJ, Kraiss LW, Galt SW. Severe coagulopathy following intraoperative use of topical thrombin. Ann Vasc Surg 2004;18(3):349–51.
[140] Spero JA. Bovine thrombin-induced inhibitor of factor V and bleeding risk in postoperative neurosurgical patients. Report of three cases. J Neurosurg 1993;78(5):817–20.
[141] Zehnder JL, Leung LL. Development of antibodies to thrombin and factor V with recurrent bleeding in a patient exposed to topical bovine thrombin. Blood 1990;76(10):2011–6.
[142] Intini G, Andreana S, Margarone 3rd JE, Bush PJ, Dziak R. Engineering a bioactive matrix by modifications of calcium sulfate. Tissue Eng 2002;8(6):997–1008.
[143] Bateman J, Intini G, Margarone J, Goodloe S, Bush P, Lynch SE, et al. Platelet-derived growth factor enhancement of two alloplastic bone matrices. J Periodontol 2005;76(11):1833–41.
[144] Intini G, Andreana S, Intini FE, Buhite RJ, Bobek LA. Calcium sulfate and platelet-rich plasma make a novel osteoinductive biomaterial for bone regeneration. J Transl Med 2007;5:13.