A Review Article:

Article review about the role of platelet rich plasma in the healing of oral tissues.

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The Biology of Platelet-Rich Plasma and Its Application In Oral Surgery

ABSTRACT

PLATELET-RICH PLASMA (PRP) is a new approach in tissue Regeneration and developing for clinicians

And researchers.. It's used in many surgical fields,

Like head and neck surgery, and oral and maxillofacial surgery.

Although the growth factors and mechanisms involved in are Still weekly understood, its possible beneficial, including bone regeneration, and rapid

Wound healing.

PRP is Prepared from the patient's own blood and contains growth factors that influence wound healing. Of

These growth factors, platelet-derived growth factor, transforming growth factor, insulin-like growth Factor, and epidermal growth factor play a pivotal role in tissue repair mechanisms its potential application in the treatment of traumatic musculoskeletal injury .

The benefit of PRP it provides a local environment for tissue regeneration which is rich in growth factors and other cytokines has been supported by vitro and animal studies which suggest a positive results. The easy application of PRP in the clinic and Its possible beneficial outcome, including reduction of bleeding, rapid soft tissuehealing, and bone Regeneration, hold promise for new treatment approaches. However, animal studies and human trials demonstrate conflicting results regarding the application of PRP.

Therefore the aim of this literature review is to evaluate the scientific evidence regarding the use of PRP in dentistry, the reported clinical use of PRP is largely confined to the last two decades and initially centred around its application in dental and maxillofacial surgery. Results from clinical studies have been encouraging.

Introduction

Research in dental and oral surgery often involves mateRials and procedures which are capable of improving Clinical outcomes in terms of percentages of success.The goal of this research was to find a treatment approach which could reduce bleeding, promote effective Bone regeneration and rapid soft-tissue healing by Employing resources which are easy to use at a modest Cost.

Platelet rich plasma (PRP) is a new approach to tissue Regeneration: it is widely used in various surgical fields, Including head and neck surgery, otolaryngology, cardio-Vascular surgery, and maxillofacial surgery. Commonly,

PRP is used in a gel formulation, which is formed by Mixing PRP (derived from the centrifugation of autolo-Gous whole blood) with thrombin and calcium chloride.

PRP gel includes a high concentration of platelets and a Native concentration of fibrinogen. During wound healing, platelets are among the first Cells to respond at a wound site, being critical to the initiation of this process. Besides their procoagulant effects, Platelets form a rich source of important growth factors,

Such as platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b) 1 and 2, and vascular endothelial growth factor (VEGF); all of these are involved in the angiogenic cascade which assists in hard and soft tissue wound healing.

Recently, PRP has become a valuable adjunct to promote healing in many procedures in dental and oral surgery. They include: ablative surgical procedures, mandibular reconstruction and surgical repair of the alveolar cleft, treatment of infrabony periodontal defects and periodontal plastic surgery, as well as procedures relating to the placement of osseointegrated implants. In such procedures, the adhesive nature of PRP facilitates the easier handling of graft material, with more predictable flap adaptation and hemostasis, and a more predictable seal than is the case with primary closure alone . Recently, the use of PRP has also been proposed in the management of bisphosphonate-related osteonecrosis of the jaw (BRONJ) or avascular necrosis, which is caused by other factors (e.g. radio-osteonecrosis), with the aim of increasing wound healing and bone maturation

Aging patients are usually the elective patients for these procedures. From a dental point of view, these patients could be considered as special needs patient, requiring a specific approach; age is considered an important determinant of periodontal disease, which is the main cause of tooth loss in adulthood. Moreover, elderly patients are mostly subject to systemic diseases which influence the response to surgical treatment in terms coagulation and tissue repair.

The improvement in quality of life of aging patients in recent decades has determined a growing request for elective treatments and devices which answer their specific needs contemporaneously.

DEFINITION AND BIOLOGICAL PROPERTIES OF PRP

According to Marx et al., PRP is an autologous concentration of human platelets above baseline in a small volume of plasma.6 However, the preparation of allogeneic PRP (aPRP; homologous) might not be excluded. Because autologous PRP preparation requires blood to be drawn from the patient, the use of aPRP may serve as an alternative option in the case of patients who refuse to be subjected to venipuncture and a blood drawing procedure. Normal platelet counts in human blood range between 150,000/mL and 350,000/mL, with an average of approximately 200,000/mL. A concentration of 1,000,000/mL in a 5-mL volume of plasma has been suggested to be the working definition of PRP, and lower concentrations may not enhance wound healing, whereas higher concentrations have not shown further enhancement of wound healing.

The properties of PRP are based on the production and release of multiple growth and differentiation factors upon platelet activation. These factors are critical in the regulation and stimulation of the healing process, and they play an important role in regulating cellular processes such as mitogenesis, chemotaxis, differentiation, and metabolism.9 However, the determinants of the biologic properties of PRP are unknown, and there is insufficient understanding of the clinical effects of PRP application.The combined action of all these growth factors is complex, and each growth factor may have a different effect on a particular tissue. Growth factors may also interact with each other, leading to different multiple signaling pathways. Different isoforms of them have varying effects that may enhance or inhibit osseous and soft tissue repair depending on the mode of growth factor release, as well as the dynamics of the wound environment.

BIOLOGICAL MECHANISM OF PRP ACTIVITY

Wound healing is a complex phenomenon related to the various growth factors and many cell types that participate in this process. Disruption of the vasculature as a result of injury leads to fibrin formation and platelet aggregation. A stable blood clot is then formed by blood coagulation. Subsequently, several growth factors are released into the injured tissue from the platelets and other cells that induce and support healing and tissue formation. PRP behaves in the same manner, and therefore the addition of thrombin and calcium chloride, resulting in the release of a cascade of growth factors from the alpha granules, activate it. The clotting process of PRP volume initiates the secretion of these growth factors, which begins within 10min after clotting.8 More than 95% of the pre-synthesized growth factors are secreted within 1h.13 After the initial burst of PRP-related growth factors, the platelets synthesize and secrete additional growth factors for the remaining 7 days of their life span. The interaction between these growth factors andsurfacess receptors on the target cells activates an intracellular signaling pathway that induces

production of proteins needed for the regenerative process (e.g. cellular proliferation, matrix formation, osteoid production, and collagen synthesis).

Additionally, PRP contains proteins such as fibrin, fibronectin, vitronectin, and thrombospondin, which are known to act as cell adhesion molecules important for osteoblast, fibroblast, and epithelial cell migration. Monoclonal antibody studies have confirmed the presence of growth factors like PDGF and TGF-b in PRP preparation. Studies have also shown that adult mesenchymal stem cells, osteoblasts,fibroblasts, endothelialcells,andepidermal cells express the cell membrane receptors that are specific to the growth factors included in PRP. Therefore, it is suggested that the growth factors included in the platelet concentrates may activate several cell types involved in wound healing and induce soft tissue healing and bone regeneration.

GROWTH FACTORS INCLUDED IN PRP

The growth factors released from the platelets include PDGF, TGF-b, platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), IGF-I, and platelet factor 4.

PDGF was first found in platelets and especially in the alpha granules. PDGF can also be found in other cells, such as macrophages, endothelial cells, monocytes, and fibroblasts, as well as in bone matrix.

Three isoforms exist: AA, BB, and AB.22 The reason for three distinct forms remains unclear, but differential binding by various receptor cells such as endothelium, fibroblasts, macrophages, and marrow stem cells has been suggested. The

most important specific activities of PDGF include angiogenesis and macrophage activation, proliferative activity on periodontal ligament fibroblasts, chemotaxis for fibroblasts, and collagen synthesis. Furthermore, PDGF enhances the proliferation of bone cells.

TGF-b has been referred to as member of a super-family of growth and differentiation factors including the bone morphogenetic proteins. TGF-b has three different isoforms: b1, b2, and b3. TGF-b has been observed to promote extracellular matrix production, to enhance the proliferative activity of periodontal ligament fibroblasts, to stimulate biosynthesis of type I collagen and fibronectin, and to induce deposition of bone matrix. TGF-b may inhibit osteoclast formation and bone resorption, thus favoring bone formation over resorption.

IGF-I is chemotactic for periodontal ligament fibroblasts and stimulates protein synthesis. IGF-I enhances bone formation by proliferation and differentiation of osteoblasts. Application of IGF-I to the surface of rat molars promotes cementogenesis, and in combination with PDGF, bone formation on implant surfaces is increased.

Cohen discovered PDEGF in 1962. It stimulates epidermal regeneration, promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts, and enhances the production and effects of other growth

Factors.

PDAF has the capacity to induce vascularization by stimulating vascular endothelial cells. Several cytokines and growth factors, including IGF-1, TGF-a, TGF-b, PDGF, PDEGF, and interleukin 1b, up-regulate PDAF.

PF-4 is also released from the alpha granules of platelets and may be partially responsible for the initial influx of neutrophils into wounds. It also acts as a chemoattractant for fibroblasts and may be a potent antiheparin agent.

REQUIRED PLATELET CONCENTRATION FOR PRP EFFICIENCY

It has been suggested that platelet concentrations ranging from 800 to 1200109 platelets/L are necessary to obtain an effective PRP dosage.39 A platelet count of 1000109 platelets/L, as measured in a volume of 5mL of plasma, may be the ‘‘therapeutic dose’’ of PRP.13 On the other hand, Anitua and coworkers stated that the platelet count of PRP should be more than 300,000/mL.40 Choi and others showed that high PRP concentrations suppressed, but that low PRP concentrations (1–5%) stimulated, the viability and proliferation of alveolar bone cells. Also, Arpornmaeklong and colleagues suggested that PRP inhibited osteogenic differentiation of pre-osteoblasts in a dosedependent manner. These reports support the view that variations in PRP concentrations may influence bone detected.

Weibrich and others examined the platelet concentration of different donors and discovered that the platelet concentration of PRP was correlated to platelet count in donor whole blood. They also found a significant effect of sex on platelet concentration, although no effect of age was detected.

Some in vitro studies have examined the effective concentration level of the growth factors included in PRP. For example, Gamal and Mailhot indicated that the optimal concentration of PDGF-BB for inducing adhesion of periodontal ligament fibroblasts to periodontitis-affected root surfaces was 50ng/mL.

Regarding the concentrations of the growth factors present in the platelet concentrations, it was demonstrated that 1mL of platelet lysates (109 platelets/ mL) contain 115ng of PDGF, 106ng of TGF-b, 20.8ng of FGF, and 0.8ng of PDEGF determined according to enzyme-linked immunosorbent assay on 35 patients. According to another study, there are approximately 0.06ng of PDGF per one million platelets.

PREPARATION OF PRP

PRP can be prepared in a laboratory or an operating or dental room from blood collected in the immediate preoperative period. Especially for dental use, the preparation of small amounts of autologous PRP can be completed in minutes and involves limited stress. Each proper PRP device must use a double centrifugation technique. The blood sample is drawn into a tube with anticoagulation factor, and then the tube is spun in standard centrifuge cycles. The first spin (hard spin) will separate the red blood cells from the plasma that contains the platelets, white blood cells, and clotting factors (buffy coat).

The second spin (soft spin) finely separates the platelet concentrate (PRP) from the platelet-poor plasma. Office devices can use only 40mL to 60mL of blood and produce approximately 5mL to 10mL of PRP. This amount is adequate for most minor surgical procedures, including bilateral sinus grafts, ridge preservation or augmentation, and periodontal regenerative therapies.2 However, in oral and maxillofacial reconstruction, up to 500mL of whole blood should be drawn to obtain the greater amounts of PRP needed for larger surgical defects.

The centrifugation process must be sterile and precisely suited to separating platelets from red blood cells and attaining adequate concentrations of platelets. Therefore, not all currently available commercial PRP devices may be the same, and some probably do not concentrate active platelets in sufficient numbers to produce a healing enhancement. This may be an explanation for the variability of the clinical efficacy of PRP. Studies suggesting that there is no benefit from PRP may be based on poor-quality PRP produced by inadequate devices. Several commercial systems are available for preparing PRP, such as the SmartPReP system (Harvest Autologous Hemobiologics, Norwell, MA), the Tisseel system (Baxter Heath Corporation, Deerfield, IL), the PCC System (Platelet Concentration Collection System, 3i Implant Innovations, Palm Beach Gardens, FL) and the Curasan PRP kit (Curasan, Pharma Gmbh AG, Lindigstrab, Germany). Appel and coworkers indicated that the PCC System and the Curasan PRP kit have greater ease of handling and shorter preparation times than the SmartPReP and the Tisseel system. Additionally, the Smart PreP system and the PCC System may produce the greatest platelet concentrations and higher levels of bioactive growth factors.

Several studies suggest different centrifugation cycles in terms of centrifugation time and force. The centrifugation force may be a critical step in PRP preparation, and the applied mechanical forces may damage platelets, with the consequence of losing the granular load of the growth factors. One study evaluated the effect of different centrifugal forces and showed that spins greater than 800g may decrease the amount of TGF-b that the PRP releases.

There are also several choices of anticoagulants that can be used during PRP preparation. Anticoagulant citrate dextrose-A is a preferable agent.8 The citrate binds calcium and prevents coagulation, whereas the dextrose and other ingredients support platelet metabolism and viability. Citrate phosphate dextrose is also useful for PRP preparation. It is similar to anticoagulant citrate dextrose-A, but it has fewer supportive ingredients and therefore may be less effective at maintaining platelet viability.

The use of ethylenediaminetetraacetic acid is potentially more harmful than citrate in the preparation of PRP, and a large number of damaged platelets have been observed. Furthermore, trisodium citrate solution is suggested as an anticoagulant with no negative effects on PRP preparation.

At the time of the application, the PRP is combined with a certain volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate) and thrombin (an activator that allows polymerization of the fibrin into an insoluble gel, which causes the platelets to degranulate and release the indicated mediators and cytokines). Specifically, Sa´nchez and colleagues proposed for the initiation of the coagulation process a mixture of 1,000UI of thrombin powder suspended in 10mL of sterile saline with 10% calcium chloride. Anitua suggested that 50mL of 10% calcium chloride should be added to a tube containing 1.2mL of PRP, and after 15 to 20min, a PRP gel can be formed without using thrombin.

IN VITRO AND ANIMAL STUDIES THAT EVALUATE THE EFFECT OF PRP

Although platelet concentrates have been used to promote bone healing, the underlying cellular-level mechanisms remain poorly understood. The effect of PRP on bone cells may not be due to the action of a single growth factor but, instead, to the synergistic effects of the many growth factors derived from platelets. It has been shown that addition of PDGF or TGF in cell cultures does not induce enhanced cell proliferation similar to that observed in the presence of a platelet concentrate. Also, applying antibodies against PDGF only partially suppressed human bone cell proliferation induced by PDGFs.

Soffer and coworkers investigated the effects of human platelet lysate (damaged platelets with microparticles of cytoplasmic and cell membrane and soluble growth factors) on rat calvaria bone cells.55 The results showed that shortterm exposure to platelet lysate (24h) promoted the proliferative and chemotactic bone cell functions, whereas long-term exposure had a negative effect, resulting in a decrease in alkaline phosphatase activity and mineral formation. Another in vitro study also showed that PRP stimulated cell growth and differentiation of rat bone marrow cells up to 8 days.

Furthermore, these results are in accordance with other reports that showed that lysed platelet solutions stimulated the proliferation of human embryonic cells and had a mitogenic effect on bone cells derived from human trabecular54 and adult rat bone marrow.

Therefore, the platelet-related activity may not be restricted to intact platelets, because microparticles of cytoplasmic and cell membrane produced from activated platelets may also stimulate human bone cell proliferation.54 These findings seem to be in disagreement with the statement of Marx's studies that platelets damaged or rendered nonviable by PRP processing may not secrete bioactive growth factors, resulting in disappointing outcomes.

However, in vitro studies use specific single cell lines with properties different from the developed cells functioning in the living human body. In addition, cell lines are often derived from origins other than human. This shortcoming simplifies the real clinical situation, in which multiple growth factors and multiple cell types interact in a complicated manner, making any extrapolation for PRP efficacy doubtful.

Furthermore, several animal studies have provided encouraging results supporting the beneficial effect of PRP on bone healing in oral surgery. The use of PRP has been reported to improve bone healing in rabbit calvaria defects and to facilitate the incorporation of particulate cancellous bone grafts in goat mandibular reconstruction. Another study assessed the efficacy of demineralized bone graft alone or combined with PRP in enhancing the osseointegration of dental implants in a dog model. The histomorphometric analysis revealed a higher percentage of bone contact in the test group. Furthermore, Nikolidakis and coworkers showed that PRP in a liquid form had a significant effect on bone apposition to roughened titanium implants during the early post-implantation healing phase in a goat model, but no effect of PRP was observed regarding the coated calcium phosphate (CaP) implants. On the other hand, there are publications that have concluded that there was little or no benefit from PRP.

For example, in an animal study, Aghaloo and others grafted 8-mm rabbit calvarial defects with autogenous bone, PRP alone, or autogenous bone and PRP; the control was no treatment. The histomorphometric evaluation showed a tendency for slightly more bone when PRP was combined with autogenous bone than for autogenous bone alone, but this difference was not significant. Also, Plachokova and coworkers showed that PRP had no effect on early bone healing in addition to an osteoconductive material (dense HA/b-TCP particles) in a rat model. Choi and others did not find any effect of PRP on bone formation in the mandible reconstruction of a canine model.69 Therefore, the analysis of the animal studies outlines the controversial nature of this issue and generates confusion regarding the beneficial effect of PRP in oral surgery.

CLINICAL STUDIES THAT EVALUATE THE EFFECT OF PRP

In addition to other medical fields, PRP can be used, alone or with bone substitutes, to promote bone healing in maxillofacial and periodontal surgery.

Anitua, in 1999, reported that the application of PRP inside the extraction sockets improved soft-tissue repair and bone Regeneration. The use of autologous platelets seemed to enhance bone autologous graft incorporation in sinus floor augmentation and showed better results in the ridge preservation grafting. De Obarrio and others combined PRP with bone allograft and guided tissue regeneration for the periodontal therapy of human intrabony defects and observed significant gain in clinical attachment, as revealed by 2-year follow-up, but no control group was present. Petrungaro, in 2001, published a case series in which PRP, subepithelial connective tissue grafts, and collagen membranes were used to cover gingival recessions; the therapy was successful in all cases.

On the other hand, in several human studies, the use of PRP with grafts in sinus elevation or in ridge augmentation did not enhance clinical results. Recent reviews have also questioned the beneficial effect of PRP in sinus augmentation. Huang and others did not observe any benefit of adding PRP for root coverage treatment in a pilot human trials. Finally, no benefit was reported after PRP application combined with bone grafts and membrane in the treatment of periodontal intrabony defects.

However, most of the human studies are case reports, and only a few randomized controlled clinical trials of PRP in oral surgery are available In summary, although the majority of these studies reported a positive effect of PRP on alveolar bone formation, this effect was minimal to modest in the most of them. Additionally, PRP may have a short time effect that extinguishes after 3 to 6 months.

RISK OF USING PRP

Because PRP is prepared from autologous blood, it is inherently safe, and any concerns of disease transmission such HIV, hepatitis, or Creutzfeldt-Jacob disease or immunogenic reactions that exist with allograft or xenograft preparations are eliminated. However, the preparation of PRP involves isolation of the PRP, after which gel formation is accelerated using calcium chloride and bovine thrombin. The use of bovine thrombin has been reported to be associated with the development of antibodies to factors V and XI, resulting in the risk of life-threatening coagulopathies. Landesberg and coworkers, in 1998, reviewed reports appearing in the literature of serious coagulopathies that were difficult to treat after exposure to bovine thrombin.

The bovine thrombin preparations contain factor V, which results in reaction of the human immune system when challenged with a foreign protein. The cross-reactivity of anti-bovine factor V antibodies with human factor V may cause the factor V deficiency after thrombin exposure. Bovine thrombin-induced coagulopathies have been reported in 32 cases of patients undergoing cardiovascular operations, specifically in patients with repeated exposure. The severity of bleeding varies widely: from no clinical evidence to life-threatening diatheses developing 7 to 14 days after successive exposure to bovine thrombin. However, the adverse reactions reported could depend upon increased awareness of the coagulopathy, as well as the source and quantity of thrombin used. Also, differences in product purity have been documented. One brand of thrombin (Thrombin-JMI, Jones Medical Industries, St. Louis, MO) applies an extra purification step to decrease the factor V concentration from 50mg/mL (until 1997) to less than 0.2mg/mL. In addition, the bovine thrombin preparations used in the reported cases were high dose (>10,000 units) and were applied directly onto open wounds where absorption into systemic circulation is certain. The use of bovine thrombin in PRP is low dose (<200 units), is topical with no entry into systemic circulation, and is already clotted when it comes into contact with human tissues. It is therefore not absorbed systemically but is engulfed and digested by the macrophages that digest the clot itself. Based on the reported coagulopathies, Landesberg and coworkers described another method of activating PRP gel with a new gelling agent (ITA, Natrex Technologies, Greenville, NC). They stated that this method could be used more safely than bovine thrombin for gelling the PRP, although they did not describe the specific composition and mechanism of action of ITA. On the other hand, some authorsproposedthat PRP gelformationcan beperformed only with theaddition ofcalcium and nothrombin is required.

The use of PRP in dental surgery

healing the alveolar socket after tooth extraction

Tooth extraction is a common dental procedure which involves severely decayed, periodontally affected, notrestorable or impacted teeth. These procedures can be associated with significant postoperative pain, particularly when third impacted molars are extracted. Furthermore, prolonged bleeding can be experienced by patients especially by those undergoing anticoagulant therapy. To address post-operative discomfort and to enhance tissue repair mechanisms, many procedures (i.e. fibrin sponge, biostimulation with LASER) have been performed which promote the healing process.

Recently, the use of PRP has been proposed as a way of obtaining high concentrations of growth factors involved in tissue healing and regeneration. The therapeutic strategy of this approach is to promote the process of tissue repair, improving

the quality of healing and healing time.

However, very few studies have been carried out on humans and contradictory results have been produced regarding the efficacy of PRP. Promising results were reported by Alissa et al. (2010), who conducted a pilot study on the effect of PRP on the healing of the hard and soft tissues of extraction sockets. Soft tissue healing was significantly improved in patients treated with PRP compared with patients of the control group (no treatment). Moreover, patients untreated with PRP experienced complications (dry sockets and acutely inflamed alveolus), which were considered to be borderline statistically significant. Radiographic evaluation revealed a statistically significant difference only for sockets with a dense homogeneous trabecular pattern. Of interest, Alissa et al. (2010) also analyzed the post-operative pain of patients of the two groups (treated and untreated) and they reported significantly more pain in the control group, especially in the first three days post intervention.

A significant response to pain in patients undergoing a surgical extraction of a single impacted third molar and using PRP was also reported by Ogundipe et al. (2011). Moreover, an improvement in swelling and the interincisal mouth opening was obtained in these patients: the scores for lamina dura, trabecular pattern, and bone density were much improved among patients in the PRP group, even if this difference was not statistically significant.

Similar findings have been reported by Ruktowski et al. (2010) who used digital radiography and Computer Tomography (CT) scan analysis to track changes in radiographic density at PRP- treated sites in comparison to ipsilateral not-PRP treated sites. The PRP- treated sites demonstrated early and a significant increased radiographic density over baseline measurements following tooth removal.

The greatest benefit attributed to PRP is during the initial 2-week post-operative healing time period: 6 weeks for control extraction sites to reach comparable bone density were required whereas PRP- treated sites achieved this at week 1. Post-operative pain and bleeding were not significantly affected by PRP application. [36]. Likewise, a more recent study by Celio-Mariano et al. (2012) showed a greater radiographic bone density in the PRP group, thereby demonstrating a significant improvement in bone healing in the sockets after extraction of mandibular third molars as compared to the control group.

In a prospective split-mouth study conducted by Arenaz-Bua et al. (2010) the efficacy of PRP in promoting bone regeneration after third molar extraction was analyzed. The Authors observed no further acceleration in bone formation at 6 months nor did they observe statistically significant differences between the groups regarding pain, swelling, trismus and infection throughout the post-operative period.

Similarly, in a study by Gurbuzer et al. (2008) (using scintigraphy), the application of PRP on its own to soft tissue impacted mandibular third molar extraction sockets failed to increase the osteoblastic activity in post-surgical weeks 1 and 4 in comparison to non-PRP-treated socket.

The above review of the literature suggests that the use of PRP in the alveolar socket after tooth extractions is certainly capable of improve soft tissue healing but there is insufficient evidence which supports the efficacy of PRP in improving bone regeneration. Similarly, the efficiency of PRP is controversial since the use of PRP in tooth extraction sites seems to influence the early phase of bone healing, thereby facilitating and accelerating bone formation in the initial period after tooth extraction, its influence decreasing after a few days. Not univocal results were also obtained for post-operative pain but conclusive considerations in terms of efficacy and efficiency could not be formulated.

The use of PRP in periodontal surgery

The growth factors present in PRP are capable of forming a fibrin clot, promoting fibroblast proliferation and upregulating collagen synthesis in the extracellular matrix. Thus, the use of PRP at injury sites might be able to promote wound healing and the regeneration of periodontal soft tissues. Moreover, the ability of these factors to accelerate bone repair by increasing the mitosis of osteoblasts and tissue vascularity might be useful in the treatment of infra-bony defects. However, the therapeutic efficacy of PRP in periodontal therapy still remains controversial.

The results of a recent systematic review regarding the efficacy of PRP in periodontal therapy have revealed that it is capable of improving gingival recession but not clinical attachment level in chronicperiodontitis.

Moreover, Pradeep et al. (2009), who conducted a study on the treatment of mandibular furcation defects, have reported the lack of complete closure of furcation defects despite a significant improvement; this implies a limited role for autologous PRP as a regenerative material.

However, the efficacy of PRP on its own is difficult to evaluate since the majority of studies have been conducted by testing PRP in combination with graft materials in order to enhance the outcome of regenerative surgery.

Moreover, a barrier membrane was used to cover the defects in most clinical studies and, thus, the effects of PRP may have been masked by the effects of the barrier.

The results of the systematic review by Del Fabbro et al. (2011) revealed that PRP may exert a positive adjunctive effect when used in combination with graft materials for the treatment of intrabony defects.

However, no significant benefit of PRP was found for the treatment of gingival recession. Similarly, two controlled clinical trials investigating the efficacy of PRP combined with other graft materials in the treatment of intraosseous periodontal defects reported a significantly more favorable clinical improvement in periodontal sites treated with the combination of PRP and the graft material than in those treated with the graft material alone. Contemporaneously, Bharadwaj et al. (2011) found that the adjunct of PRP to bone graft appeared to be beneficial in the treatment of human periodontal intrabony defects.

Different results have been reported by other Authors, who showed no significant benefits regarding the additional use of PRP to graft materials in the treatment of infra-bony defects. Ozdemir et al. (2012) showed that PRP combined with a graft material was effective in the treatment of intrabony defects after a 6-month healing period but no additional statistically significant improvements were observed when PRP was used.

Similar results were reached by Harnack et al. (2009) using the same combination of materials. Rodrigues et al. (2011) concluded that both PRP and PRP combined with bovine anorganic bone mineral (ABM) resulted in a significant clinical improvement for the treatment of human periodontal intrabony defects but there was a preponderance of improved clinical results with the addition of ABM to PRP. No additional effects were found by Döri et al. (2008, 2009) and Piemontese et al. (2008). Throughout all their studies, they concluded that the use of PRP failed to enhance the results obtained with the use of the graft material used on its own

Few clinical comparative studies have investigated the use of PRP in the treatment of gingival recession. Keceli et al. (2008) did not reveal promising results and nor did they observe differences in clinical outcomes between connective tissue grafts (CTG) and a CTG-PRP combination. The results of this analysis reflect the limited and heterogeneous data available and suggest that the specific selection of agents/procedures combined with PRP could be important.

The employ of PRP in oral surgery

The use of PRP in soft tissues and bone tissues surgery and implant surgery

Animal and human studies have demonstrated that PRP enhances and accelerates soft tissue repair and bone Regeneration. In the field of bone tissue surgery, a recent study by Daif (2012) investigated the effect of autologous PRP on bone regeneration in mandibular fractures. He concluded that direct application of the PRP along the fracture lines may enhance bone regeneration. Wojtowicz et al. (2007) compared the effects of stimulating the osteogenesis of the alveolar bone by transplants of autologous bone marrow and freshlyisolated mononuclear cells from bone marrow, containing CD34+ cells and PRP. It was shown that newlyformed bone increased under the influence of PRP. This treatment was more effective than that using the population of CD-34 bone marrow- derived stem cells

A Cochrane review of Esposito et al. (2010) concluded that PRP treatment did not seem to improve the clinical outcome of sinus lift procedures with autogenous bone or bone substitutes. In addition, a study by Khairy et al. (2012) evaluated the bone quality in sinus which had been augmented with autogenous bone with or without PRP. The conclusion was that enrichment with PRP did not significantly improve bone density at 3 months post grafting but PRP- enriched bone grafts were associated with superior bone density at 6 months post grafting. Poeschl (2012) obtained successful results when PRP was used in combination with a graft material in maxillary sinus augmentation. Cabbar (2011) et al. Compared a bovine bone xenograft with PRP and without PRP to augment the human maxillary sinus in preparation for receiving dental implants. The conclusion of this study stated was that the combination of the xenograft and PRP did not have any effect on new bone formation and implant stabilization

The preparation of PRP, as applied to an implant surface, adheres to metal and might create a new dynamic surface which could potentially show biological activity. In 2006, Anitua showed that the osseointegration of implants was enhanced by coating the implant surface with PRP prior to insertion into the alveolus. Similarly, Gentile et al.(2010) reported their experience on 15 cases, including reconstructive surgery of the jaw, post-extraction alveolar bone regeneration, and oral implantology. The results of their study revealed the efficacy of the PRP treatment in terms of post-operative patients' satisfaction and low-morbidity. Anand et al. (2012) have recently proposed that the use of a novel technique (of coating the implant with PRP) could improve the prognosis of the treatment regarding an immediate loading protocol

The results of these studies demonstrate that PRP is effective in soft tissue healing and bone regeneration. The combination of PRP application with other biomaterials seems to be promising as regards sinus lifting, but the results depend on the material used. Promising results have also been obtained in implant surgery, using PRP on its own as a coating material.

The use of PRP in BRONJ surgery

Some researchers have proposed the use of PRP in BRONJ surgery. BRONJ is currently recognized as a significant complication, which is related to the use of bisphosphonates (BPs), a widely-used class of drugs employed in the preventative treatment of various pathologies joined by the same alteration of bone turn-over (i.e. osteoporosis, bone metastasis, associated with solid tumours and multiple myeloma, malignant hypercalcemia). BPs are capable of inhibiting osteoclast-mediated bone resorption, also displaying anti-angiogenetic activity. The bones of patients treated with BPs are, therefore, poorly vascularized and poorly supplied with the substances necessary for wound healing.

The use of these drugs could delay the onset of BRONJ, which is defined as an avascular area of necrotic bone in the maxillofacial area, with or without exposed bone. This area of osteonecrosis always appears in the traumatized bone. Although some of the cases reported were asymptomatic, most of them resulted in complications, such as an altered sensation in the affected area (e.g. mandibular alveolar nerve), purulent exudates, oralcutaneos fistula, and mandibular fractures. BRONJ management is currently controversial, ranging from medical to surgical treatment, with no definitive standard of care. Indeed, the response to radical surgery is less predictable than in other situations involving bone necrosis, such as radiotherapy or osteomyelitis. Aggressive surgical debridement is also controversial due to the risk of worsening bone exposure. Occasionally, the bone is left exposed due to the difficulty of treating the lesion.

PRP therapy has been proposed as a complement to conservative surgery in order to enhance bone healing. The rationale for the employment of PRP in patients affected by BRONJ is based on the thesis that the presence of growth factors (usually repressed by BPs) constitutes a substitute stimulation to bone healing, which is similar to physiological healing. The growth factors in PRP might accelerate epithelial wound healing, decrease tissue inflammation after surgery, improve the regeneration of bone and soft tissues, and promote tissue vascularization. The additional advantages related to the use of this product are its biocompatibility and safety, as an autologous product

Few case series studies relating to use of PRP in treating BRONJ have been published: Cetiner et al., 2009 described a case of zoledronate-associated BRONJ after tooth exodontia in a 68-year-old man with multiple myeloma, which was treated with surgical debridement plus PRP with a positive outcome after a 6-month follow-up period. Curi et al., 2007 reported using this treatment in three cases of jaw lesions, which were followed up for 6 months in two cases and 8 months in one.

Lee et al., 2007 reported two cases which were successfully treated, which were secondary to complications of dental implants: one involved left oral sinus communication (with a 9-month follow- up period) and the other case involved a lesion on the left jaw ramus (6-month follow-up).

In the study conducted by Bocanegra et al. (2012) eight patients were selected and all improved over a mean period of 3 weeks (2–4 weeks) after treatment with: fast mucosal healing, a reduced need for analgesics and a resolution of mouth lesions. These patients continued with follow-up visits, without any evidence of exposed bone after 14 months. Adornato et al. (2007) treated twelve patients who presented with soft tissue ulcerations and bone exposure with measurements ranging from 5 to 25 mm. These lesions had not responded to six months of treatment with cleaning therapies, 0.12% chlorhexidine rinses and intermittent antibiotic therapies. These patients were treated with conservative marginal resections of the alveolar bone with primary closure over the bony defect, PRP and a resorbable membrane under antibiotic coverage. After six months, ten patients showed complete soft tissue healing with one patient displaying a recurrence of epithelial dehiscence; another patient, with recovery by secondary intention, did not show any regression of bone exposure.

Positive results were also obtained by Mozzati et al. (2012), who conducted a study on 32 patients treated with intravenous BPs for oncological pathologies affected by BRONJ. The patients were treated by a resection of the necrotic bone with primary closure of the mucosa over the bony defect using PRP. The orthopanoramic and computed tomography performed before and after surgery revealed successful outcomes. Similarly Coviello et al. (2012), who reported the cases of 7 patients with multiple myeloma treated with BPs, concluded that the use of PRP enhances wound healing and reduce bone exposure and would be an effective treatment protocol to use in BRONJ subjects. The results of these studies showed that the combination of necrotic bone curettage and PRP application seem to be promising for the treatment of refractory BRONJ. Since an efficient standard treatment has not yet been established, this combined approach can be considered a treatment option as it has demonstrated successful outcomes and minimal invasivity.

The risk/benefit ratio and the use of PRP

PRP is an autologous preparation, utilizing the patient's own blood in a significantly small quantity. For this reason, it is safe and there have been no published references relating to the risk of infections, disease transmission (such as HIV, hepatitis, or Creutzfeldt-Jacob disease), immunogenic reactions or any other adverse effects which exist with allografts or xenografts.

In the past, the use of bovine thrombin (an activator which allows polymerization of fibrin into an insoluble gel), used in the preparation of PRP, was associated with the risk of life-threatening coagulopathies. However, the adverse reactions reported were related to the source and quantity of thrombin used. The use of bovine thrombin in PRP in low doses (<200 units), topically with no entry into systemic circulation and already clotted when coming into contact with human tissues, would not be dangerous as an immunologic reaction. Moreover, in the second generation platelet concentrate, PRP activation was effected using only calcium chloride, thus eliminating the risk associated with thrombin. No adverse effects linking the use of calcium chloride have been reported in the literature.

Although no undesirable effects have been reported in the many clinical cases subjected to PRP therapy, hypotheses as to the over-expression of growth factors and their receptors related to tumour and dysplastic tissues have been postulated. These hypotheses are founded on the fact that growth factors appear to regulate different cellular processes, such as mitogenesis, chemotaxis, cell differentiation and metabolism.

However, the phenomenon leading to neoplastic growth requires more continuous doses of growth factors over time than those applied in PRP therapy and sufficient delivery, taking into account that extracellular growth factors degrade within 7–10 days.

Moreover, there are previously existing alterations for developing a neoplasm and, in any case, the use of PRP should be avoided: in patients with precancerous oral conditions and in the vicinity of precancerous lesions (oral leukoplakia, erythroplasia or solar cheilitis); areas of oral epithelial dysplasia; and in patients with a prior history of exposure to carcinogens or primary oral squamous cell carcinoma.

The only disadvantage of PRP preparations would be the cost versus the outcome benefit. The doubtful success of PRP may not justify the cost to the clinician of buying the PRP-processing system and the disposable kits or the cost to the patient for paying for this treatment. Furthermore, an additional but less important inconvenience for the treatment would be that patients have to be subjected to a venipuncture and blood drawing procedure in preparation for pation.

On the other hand, PRP has the advantage to being easily obtainable and not time-consuming for the patient and/or clinician.

Even if preparation of PRP involves an additional step to the surgical procedure, it takes approximately 30 minutes and is best performed by a surgical assistant under the supervision of a trained dental surgeon. This can be done simultaneously while performing the surgery, and it, therefore, does not significantly increase the chair time of the operator and the patien.

Conclusion

PRP preparations have been proposed for several uses in dental and oral surgery. The ease with which these preparations are used might be helpful to the dental professional in many surgical procedures, and their safety might encourage their wide employment. The scientific evidence regarding the efficacy and efficiency of PRP is still controversial, given the paucity of RCTs relating to this topic and, of these, the majority has been conducted using different graft materials and applying different procedures.

This Review of the literature suggests that the use of PRP in the alveolar socket after tooth extractions is certainly able to improve soft tissue healing and positively influence bone regeneration but this latter effect seems to decrease a few days after extraction.

PRP has revealed better results in periodontal therapy in association with other materials than when it is used alone, suggesting that the specific selection of agents/procedures combined with PRP could be important.

Promising results have also been obtained in implant surgery, using PRP on its own as a coating material. Furthermore, the combination of PRP application with other biomaterials seems to be favorable as regards sinus lifting even if the choice of material used is critical in this field. The combination of necrotic bone curettage and PRP application seem to be encouraging for the treatment of refractory BRONJ, as it has demonstrated successful outcomes with minimal invasivity. Since it is free from potential risks to patients, not difficult to obtain and use, PRP can be employed as a valid adjunct to many procedures in oral and dental surgery. However, further RCTs are required to support the use of PRP in current practice.

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