Review: Application of Platelet-Rich Plasma in Hard Tissue Defects

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Abstract

Platelet Rich Plasma (PRP) derived from autologous whole blood is a platelet concentrate. After its first clinical application in 1987 by Ferrari et al. [1] in an open heart surgery, used to avoid excessive transfusion of homologous blood products, the application of autologous PRP has been safely used and documented for 20 years in many fields including: maxillofacial surgery [2,3] aesthetic plastic surgery [5-7] treatment of soft-tissue ulcers [16,17] up to regenerative surgery.

This article introduces the reader to PRP therapy and reviews the current literature on this emerging treatment modality, showing at the current state, the clinical use of PRP in hard tissue defects with innovative methods and future prospects.

However there is a great debate in the scientific community on appropriate protocols of PRP application.

In summary, PRP provides a promising alternative to surgery by ensuring safe and natural healing.

Keywords: Platelet-Rich Plasma; Stem cells; Reconstructive surgery; Hard tissue defect

Introduction

The concept of Platelet Rich Plasma (PRP) started in 1975 with CJ. Oon and JR. Hobbs [4] with Continuous Flow Blood Separator Machine for the selective removal or exchange of either packed red blood cells, leucocyte-rich or platelet-rich layers or plasma. Although, various platelet gfs had been discovered by then, the clinical use of activated PRP was rarely reported in the 1980s.

After its first clinical application used in 1987 by Ferrari et al. [1] in an open heart surgery, to avoid excessive transfusion of homologous blood products, the autologous PRP has been safely used and documented for 20 years in many fields including: maxillofacial surgery [2,3] aesthetic plastic surgery [5-7] treatment of soft-tissue ulcers [8,9] up to regenerative surgery. Infact, PRP had a great increase in popularity after 1998 and it is now widely accepted that the correct use of PRP in tendon healing [11], Orthopedic Sport Injuries [12], dental implants [13] and Chronic Cutaneous Ulcers [14]. In 2009 a study previously determined [16].

In 1999 Anitua E [10] reported preliminary clinical evidence of the beneficial effect of the use of autologous plasma rich in growth factors in bone regeneration using plasmapheresis.

After the publication of a large number of articles about the use of PRP in tendon healing [11], Orthopedic Sport Injuries [12], dental implants [13] and Chronic Cutaneous Ulcers [14]. In 2009 a study about fibroblastic response to treatment with different preparations rich in growth factors [15] was reported.

This article is a focused review of the clinical approaches to the use of PRP describing methods of updated application. There is a large study in this area, which dates back 20 to 30years. In this review, there is a focus on the advances that have been made in the last decade. Our intent is not to neglect the important contributions of the pioneers in this field but rather to provide a concise update of recent work in this area. For readers who already are in the field of platelet rich plasma, we hope this review will provide a concise compilation of recent advances and the context in which they were made.

Platelet Rich Plasma Preparation

How is PRP currently prepared?

There are a large number of methods on the preparation of PRP. The traditional preparation consisted in a slow centrifugation, which allows the platelets to remain suspended in the plasma, while the leukocytes and erythrocytes are displaced to the bottom of the tube. A rapid centrifugation can cause mechanical forces and can raise the temperature, thus inducing changes in the ultra structure of platelets that, in turn, can initiate a partial activation, with a consequent loss of its content [16].

The current systems for preparing platelet concentrations uses various centrifuges (the authors use 1100 g for 10 min). The authors prepared PRP from a small volume of blood (18 cc) according to the method of Cascade-Fibrinet-Esforax system [16] with modifications. Briefly, to prepare PRP, blood was taken from a peripheral vein using sodium citrate as an anticoagulant.

Although the preparation is not selective, the final aim was to obtain a platelet pellet that included leukocytes.

The secretion of growth factor begins with platelet activation. This PRP protocol uses Ca2+ to induce platelet activation and exocytosis of the granules. Calcium acts as a necessary cofactor for platelet aggregation [16]. When Ca2+ is used to induce platelet activation, the secretion of the growth factors contained in the granules is slow. To optimize the secretion process, the optimum concentration of Ca2+ was previously determined [16].

References

[1] Ferrari et al. 1987
[5] Stem cells
[6] Reconstructive surgery
[7] Hard tissue defects
[8] PRP
[9] Ca2+
[12] Orthopedic Sport Injuries
[13] Dental implants
[14] Chronic Cutaneous Ulcers
[16] PRP
After centrifugation, theuffy coat layer, consisting of platelets and white blood cells, was placed in a volume of 9 cc of plasma.

Anitua et al. Reported: “Blood was collected into 3.8% (wt/vol) sodium citrate from six healthy donors. Samples were centrifuged either at 4500g for 12 min at 4°C to obtain PP-plasma or at 460g for 8 min to obtain PR-plasma. Platelet counts were performed with a Beckman-Coulter act Differential Analyzer (Galway, Ireland) in both the PP-plasma and PR-plasma before clotting.

Calcium chloride was added to PP- and PR-plasma of each donor at a final concentration of 22.8 mm. After calcification, 150_L of either PP- and PR-plasma were immediately dispensed onto 48 well plates; all fibrin matrices were formed “in situ” within 15 min, covering the whole surface of the wells. Control cultures were set using purified human fibrogenin (3.5 mg/ml, Calbiochem, Darmstadt, Germany) that was converted into fibrin by addition of 1.5 U of human Thrombin (Stago, France) and 22.8 mm calcium chloride” [17].

Are the methodological changes to the preparation of PRP in literature?

When autologous blood is centrifuged, three layers form because of its density: the bottom layer consisting of red blood cells (specific gravity, 1.09), the middle layer consisting of platelets and white blood cells (buffy coat; specific gravity, 1.06), and the top plasma layer (specific gravity, 1.03) [18]. Centrifugation forms the basis of current methods for producing platelet-rich plasma, with the yield approximately 10% by volume, of whole blood drawn. Platelet fragmentation during processing should be avoided.

Standard cell separators and salvage devices can be used to produce platelet-rich plasma. These devices operate on a unit of blood and typically use continuous-flow centrifuge bowl or continuous-flow disk separation technology and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations from two to four times baseline [19,20]. Such devices include the CATS (Fresenius, Wilmington, Del.), Sequestra (Medtronic, Minneapolis, Minn.), Haemonetics Cell Saver 5 (Haemonetics Corp., Braintree, Mass.), and others [19,20].

Many surgical procedures require the use of relatively small volumes of platelet-rich plasma [21]. Consequently, small, compact office systems have been developed producing approximately 6 ml of platelet-rich plasma from 45 to 60 ml of blood [21-23]. There are many systems, including the GPS (Biomet, Warsaw, Ind.), the PPCS (Implant Innovations, Inc., Palm Beach Gardens, Fla.), the Symphony II (DePuy, Warsaw, Ind.), the SmartPreP (Harvest Technologies Corp., Norwell, Mass.), and the Magellan (Medtronic, Minneapolis, Minn.) [19,22-24]. Although all operate on a small volume of drawn blood (45 to 60 ml) and on the principle of centrifugation, these systems differ widely in their ability to collect and concentrate platelets, with approximately 30 to 85% of the available platelets collected and from a less than 2-fold to an approximately 8-fold increase in the platelet concentration over baseline [19,20].

The authors used the Cascade-Fibrinet (Cascade Medical Enterprises, Plymouth, Devonshire, UK), Vivostat (Vivostat A/S, Borupvang 2, DK-3450 alleroed, Denmark), Regen (Regen Lab, En Budron B2, CH-1052 Le Mont-sur-Lausanne, Switzerland).

Generally, most systems, whether large or small volume, do not concentrate the plasma proteins of the coagulation cascade [19,24]. The concentration of plasma protein levels above baseline can be achieved through secondary ultrafiltration, as in the ultraconcentrator (Interpore Cross, Irvine, Calif.), and the Access System (Interpore Cross), in which the buffy coat collected from a centrifugation stage is passed through hollow fibers with an effective pore size of 30 kDa. With this system, up to two-thirds of the aqueous phase is removed by filtration; thus, the concentrations of the retained plasma proteins and other elements are increased [25,26].

**PRP clinical application in hard tissue defect**

The authors feel that there are new issues in literature about the selection of the most appropriate regenerative methods based on the use of a PRP. Indeed, there are many publications regarding the use of PRP with or without scaffolds in plastic and reconstructive surgery and in maxillofacial surgery; The circulating platelets participate in natural wound healing based on its numbers in circulating blood and activation status in response to [27-30] factors present in blood.

The activation process causes significant structural and morphological changes in platelets. Granules migrate to the surface of platelets and fuse with platelet-membrane creating an active secretion of GF that are store in these granules, which results in wound healing. Healing of hard and soft tissues is mediated by a complex array of intracellular and extra-cellular events that are regulated by signaling proteins. The higher concentrations of GF are present in PRP-bone graft material mix and have a greater response for osteogenesis compared to a physiological blood clot. There is evidence that show an increase in vascularization in the first 20 days followed by an increase in osteoblast activity, which forms immature bone or osteoid tissue within 3-6 weeks. There is a constant maturation by increased osteoblast activity to form a harder and mature bone [18] on a cellular level, PRP has shown to effect positively osteoblasts and fibroblasts. However, its effect is more osteogeneic rather than osteoinductive. [32,33] Marx [32,34] stresses the fact that PRP is beneficial with autogenous rather than alloplastic or xenogenic grafts. In a study for mandibular reconstruction the degree of maturation of grafts was significantly greater with the addition of PRP (1.6 to 2.2 times) [35]. In the same study, a greater percentage of trabecular bone was found histomorphometrically with the addition of PRP (74%) than without PRP (55%). This has been the main argument explaining why some studies with non-vital bone have shown no results. However, other studies showed that PRP enhances bone-forming function not only of autogenous bone but also of bone grafting materials by reducing healing time, enhancing wound healing, improving bone quality and reducing donor site morbidity [36-38].

Of the 11 trials assessing the use of PRP in oral and maxillofacial surgery [39-43] had a parallel design, one study did not specify the study design, and five studies [44-48] followed a “split-mouth” design. Although all studies were randomized, none explained the process of allocation to treatment as blinded to investigator (e.g., allocation by a central office unaware of subjects’ characteristics or sequentially numbered, sealed, opaque envelopes). In the studies with split-mouth design [44-48] the treatment was located in a specific side of the mouth based on a random criterion. Three studies [43-45] were double blind and in another [42,46,47] the evaluator was blinded to intervention. The other 5 studies [39-41,48] were not blinded.

The numbers of patients included in each trial were relatively small (n<100) and no trial reported case random sample size calculation. Overall, the quality of five studies [39-41,48] was low and moderate [42-47] Five Randomized Clinical Trials (RCTs) [43-46] were conducted in patients with intra-bone periodontal defects secondary to chronic periodontitis, three RCTs were performed with patients WHO needed sinus floor augmentations with or without implants.
Two studies [39,41] were conducted in patients with indicated dental extractions, and another study was conducted in patients with maxillary bone grafts in mandibular defects secondary to extirpation of a benign or malignant tumor.

It was possible only to combine the results of 4 of 11 RCTs [42-45] excluding one RCT [47] all of the non-meta-analyzed studies concluded that the PRP group showed better results than the control group. Only one, [45] of them specified the p value.

The results of four studies [42-44,46] were combined to analyze the efficiency of PRP in “depth reduction of gingival recession” (mm) versus a control group in patients suffering from chronic periodontitis. The mean effect showed a greater reduction in the experimental group (153 patients; SMD, 0.54mm; range, 0.16-0.92 mm).

Three studies [43,44,46] assessing the “clinical attachment level” (mm) showed non-significant differences between the experimental and control groups (126 patients; SMD, 0.33 mm; range, -0.71 to 1.37 mm), and an important heterogeneity was observed (I² = 86%). The heterogeneity was explained by the differences in the crPRP disease severity among studies. Results were homogeneous (I² = 0%) only when [43,44] patients with severe stages were considered, and were favorable to the experimental group (96 patients; SMD, 0.89 mm; range, 0.47-1.31 mm).

Diabetes impairs fracture healing with reduced early proliferation of cells, delayed osteogenesis, and diminished biomechanical properties of the fracture callus [49,50]. In an animal study by Gandhi et al., male Wister rats received closed mid-diaphyseal fractures after 14 days of the onset of diabetes. PRP did not alter blood glucose levels or HbA1c. The study demonstrated that diabetic rats had decreased growth factors compared to non-diabetic group [34].

However, not all studies on autologous growth factors have shown favorable results with promoting bone formation and healing. In a recent study by Ranly et al., PRP was shown to decrease osteoinductivity of demineralized bone matrix in immunocompromised mice. PRP from six healthy men was implanted as gelatin capsules in the calves of inbred nude mice. After 56 days the mice were killed and the studied calf muscles suggest that PDGF may actually reduce osteoinductivity of demineralized bone matrix in immunocompromised mice.

The study demonstrated that diabetic rats had decreased growth factors compared to non-diabetic group [34].

References
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