Review

Platelet Concentrates:
A Promising Innovation In Dentistry

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Abstract:

A recent Innovation in dentistry is the preparation and use of Platelet Concentrates (PRP, PRF), a concentrated suspension of growth factors found in platelets. These growth factors are involved in wound healing and are postulated as promoters of tissue regeneration. This article describes the methods of preparation, clinical application and safety concerns of PRP and also the evolution of second generation platelet concentrate, referred to as PRF.

Key Words: Platelet rich plasma, growth factors, Platelets.

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Introduction :

One of the last achievements in dentistry is the use of platelet concentrates for the improvement of reparation and regeneration of the soft and hard tissues after different surgical procedures. Post surgically, blood clots initiate the healing and regeneration of hard and soft tissues. Using platelet concentrates, is a way to accelerate and enhance the body's natural wound healing mechanisms. A natural blood clot contains mainly red blood cells, approximately 5% platelets and less than 1% white blood cells[1]. It is now well known that platelets have many functions beyond that of simple hemostasis. Platelets contain important growth factors

that initiate and support wound healing.

Platelet Concentrates: Evolution:

In general, platelet concentrates are blood derived products used for the prevention and treatment of hemorrhages due to serious thrombopenia of the central origin. The development of platelet concentrates as bioactive surgical additives that are applied locally to promote wound healing stems from the use of fibrin adhesives. Since 1990, medical science has recognized several components in blood, which are a part of the natural healing process; when added to wounded tissues or surgical sites, they have the potential to accelerate healing. Fibrin glue was originally described in 1970 and is formed by polymerizing fibrinogen with thrombin and calcium. It was originally prepared using donor plasma; however, because of the low concentration of fibrinogen in plasma, the stability and quality of fibrin glue were low [2].

It is now well known that platelets have many functions beyond that of simple hemostasis. Platelets contain important growth factors that, when secreted, are responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular in-growth, and inducing cell differentiation[3].

The study of these growth factors combined with the discovery of their extrusion by platelets has led to the development of an autologus platelet gel – PRP – to be used in various surgical fields. Whitman et al have called PRP an "autologus alternative to fibrin glue". Fibrin glue obtained through blood bank donations has been used for years as hemostatic agent and surgical adhesive. The important difference in composition between PRP and Fibrin Glue is the presence of a high concentration of platelets and

native concentration of fibrinogen in PRP[4].

The present paper describes the preparation and uses of two commonly used platelet concentrates: Platelet-rich plasma (PRP) and Platelet rich Fibrin (PRF).

Platelet Rich Plasma:

Platelet rich plasma (PRP) is an autologus concentrate of human platelets in a small volume of plasma. Therefore, the term PRP is preferred to autologus platelet gel, plasma-rich growth factors (PRGFs) or a mere autologus platelet concentrate [5].

Platelet rich plasma was developed in the early 1970's as a byproduct of multi-component pheresis[6]. Techniques and equipment have dramatically improved through the 1990's. However, the credit of introducing platelet-rich plasma into contemporary oral surgery goes to Whitman et al who first advocated its use for oral surgical procedures in 1997 [4].

Preparation of PRP:

PRP can be prepared by two techniques.

1. General-purpose cell separators
2. Platelet-concentrating cell separators

1. General-purpose cell separators:

It requires large quantities of blood (450 ml) and generally requires to be operated in a hospital setting. Blood is drawn into a collection bag containing citrate-phosphate-dextrose anticoagulant. It is first centrifuged at 5,600 rpm to separate RBCs from platelet-poor plasma (PPP) and PRP. The centrifugation speed is then reduced to 2,400 rpm to get a final separation of about 30 ml of PRP from the RBCs. With this technique, the remaining PPP and RBCs can be returned to the patient's circulation or can be discarded. The ELMD-500 (Medtronic Electromedic, Auto Transfusion System, Parker, CO,

USA) cell separator is widely used for this technique.

2. Platelet-concentrating cell separators:

It requires small quantity of blood and can be prepared by using certain equipments in a dental clinic set up. Currently, two such systems are approved by FDA and commercially available: Smart PreP (Harvest Technologies, Plymouth, MA, USA) and the Platelet Concentrate Collection System (PCCS; 3i Implant Innovations, Inc, West Palm Beach, FL, USA). Several studies have been performed to compare the efficacy of these systems [7-9]. A study conducted by Marx et al[10] indicated that of all of the devices tested these 2 FDA cleared PRP devices produced greatest platelet concentrates and most important, release of therapeutic level of bio-active growth factors.

The preparation and processing of PRP is quite similar in most of the platelet-concentrating systems although the anticoagulant used and the speed and duration of centrifugation may differ with different systems.

1. Venous blood is drawn into a tube containing an anticoagulant to avoid platelet activation and degranulation.
2. The first centrifugation is called "soft spin", which allows blood separation into three layers, namely bottom-most RBC layer (55% of total volume), topmost acellular plasma layer called PPP (40% of total volume), and an intermediate PRP layer (5% of total volume) called the "buffy coat".
3. Using a sterile syringe, the operator transfers PPP, PRP and some RBCs into another tube without an anticoagulant.
4. This tube will now undergo a second centrifugation, which is longer and faster than the first, called "hard spin". This allows the platelets (PRP) to settle at the bottom of the tube with a very few RBCs, which explains the red tinge of the final PRP

preparation. The acellular plasma, PPP (80% of the volume), is found at the top.

5. Most of the PPP is removed with a syringe and discarded, and the remaining PRP is shaken well.
6. This PRP is then mixed with bovine thrombin and calcium chloride at the time of application. This results in gelling of the platelet concentrate. Calcium chloride nullifies the effect of the citrate anticoagulant used, and thrombin helps in activating the fibrinogen, which is converted to fibrin and cross-linked[11].

Mechanism of action of PRP:

PRP works via the degranulation of the α granules in platelets, which contain the synthesized and pre-packed growth factors. The growth factors which are released from activated platelets were:

1. Platelet derived growth factor (PDGF)
2. Transforming growth factors beta 1 and beta 2 (TGF β 1 & 2)
3. Vascular Endothelial Growth Factor (VEGF)
4. Platelet derived endothelial cell growth factor
5. Interleukin – 1 (IL-1)
6. Basic fibroblast growth factor (bFGF)
7. Platelet activating factor -4 (PAF-4)

The active secretion of these growth factors is initiated by the clotting process of blood and begins within 10 minutes after clotting. More than 95% of the presynthesized growth factors are secreted within 1 hour. Therefore, PRP must be developed in an anticoagulated state and should be used on the graft, flap, or wound, within 10 minutes of clot initiation [12, 13].

The secreted growth factors immediately bind to the external surface of cell membranes of cells in the graft, flap, or wound via transmembrane receptors. These

transmembrane receptors in turn induce an activation of an endogenous internal signal protein, which causes the expression of (unlocks) a normal gene sequence of the cell such as cellular proliferation, matrix formation, osteoid production, collagen synthesis etc. thus PRP growth factors act through the stimulation of normal healing, just much faster[14,15].

Clinical applications of PRP:

Because PRP enhances osteoprogenitor cells in the host bone and in bone grafts[12], it has found clinical applications in.

1. Continuity defects[12,16]
2. sinus lift augmentation grafting[17,18]
3. Horizontal and vertical ridge augmentations[19]
4. Ridge preservation Graftings[20]
5. Periodontal/peri- implant defects[21]
6. Cyst enucleations/Periapical surgeries
7. Healing of Extraction wounds
8. Endodontic surgeries and Retrograde procedures
9. Ablative surgeries of the Maxillo-Facial region
10. Blepharoplasty

Safety concerns of PRP:

Because it is an autogenous preparation, PRP is inherently safe and therefore free from concerns over transmissible diseases such as HIV, Hepatitis, West Nile fever, and Cruetzfeld-jacob disease (CJD) ("mad vow disease") [10].

However, Sanchez *et al* [22] have elaborated on the potential risks associated with the use of PRP. The preparation of PRP involves the isolation of PRP after which gel formation is accelerated using calcium chloride and bovine thrombin. It has been discovered that the use of bovine thrombin may be associated with the development of antibodies to the factors V, XI and thrombin, resulting in the risk of life-threatening coagulopathies. Bovine

thrombin preparations have been shown to contain factor V, which could result in the stimulation of the immune system when challenged with a foreign protein. Marx et al[10] in their article stated that the second set of bleeding episodes in the patients who developed coagulopathies were not due to antibodies against bovine thrombin or human thrombin but instead due to antibodies that developed to bovine factor Va that was a contaminant in certain bovine thrombin commercial preparations.

Other methods for safer preparation of PRP include the utilization of recombinant human thrombin, autologous thrombin or perhaps extra-purified thrombin. Landesberg *et al* [23] have suggested that alternative methods of activating PRP need to be studied and made available to the dental community.

Platelet rich Fibrin (PRF):

PRF was first developed in France by Choukroun *et al*. It has been referred to as a second-generation platelet concentrate, which has been shown to have several advantages over traditionally prepared PRP. Its chief advantages include ease of preparation and lack of biochemical handling of blood, which makes this preparation strictly autologus[24].

Preparation

The preparation of PRF is very simple. Since, bovine thrombin is not used for the preparation; PRF is free from associated risks. The required quantity of blood is drawn into 10-ml test tubes without an anticoagulant and centrifuged immediately. Blood is centrifuged using a tabletop centrifuge for 12 min at 2,700 rpm. The resultant product consists of the following three layers:

- Topmost layer consisting of acellular PPP
- PRF clot in the middle
- RBCs at the bottom

Because of the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful preparation of PRF, speedy blood collection and immediate centrifugation, before the clotting cascade is initiated, is absolutely essential[24]. PRF can be obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

PRF is in the form of a platelet gel and can be used in conjunction with bone grafts, which offers several advantages including promoting wound healing, bone growth and maturation, graft stabilization, wound sealing and hemostasis, and improving the handling properties of graft materials. PRF can also be used as a membrane. Clinical trials suggest that the combination of bone grafts and growth factors contained in PRP and PRF may be suitable to enhance bone density. In an experimental trial, the growth factor content in PRP and PRF aliquots was measured using Elisa kits. The results suggest that the growth factor content (PDGF and TGF-β) was comparable in both. Another experimental study used osteoblast cell cultures to investigate the influence of PRP and PRF on proliferation and differentiation of osteoblasts. In this study, the affinity of osteoblasts to the PRF membrane appeared to be superior[22].

PRF has many advantages over PRP. It eliminates the redundant process of adding anticoagulant as well as the need to neutralize it. The addition of bovine-derived thrombin to promote conversion of fibrinogen to fibrin in PRP is also eliminated. The elimination of these steps considerably reduces biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. The conversion of fibrinogen into fibrin takes place slowly with small quantities of physiologically available thrombin

present in the blood sample itself. Thus, a physiologic architecture that is very favorable to the healing process is obtained due to this slow polymerization process.

Literature pertaining to PRF is found in French, and the material is being used widely in France. The popularity of this material should increase considering its many advantages. The findings of Wiltfang *et al* [25] from a series of clinical trials are encouraging, in that they show improved properties of PRF as compared with PRP. In future, more histologic evaluations from other parts of the world are required to understand the benefits of this second-generation platelet concentrate.

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