Review Article

Autologous Platelet Concentrate Preparations in Dentistry

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Abstract

The application of autologous platelet concentrates (APCs) in the field of regenerative procedures remained enigma to majority of the clinical practitioners. Platelet concentrates are the blood derivatives which are prepared from the patient's own blood, in which the activated platelets were entrapped within a scaffold of fibrin matrix and intended to release the growth factors and cytokines involved in the key processes of tissue regeneration which includes cell proliferation, differentiation, extracellular matrix synthesis, chemotaxis, as well as angiogenesis. These processes can accelerate and promote healing of both soft and hard tissues finding its role successful in the field of medical and dental fraternity. Hence, the biology behind, the protocols for preparation, and the science-based evidence in the applications of various platelet concentrates in many fields of dentistry will be reviewed in this literature briefly.

Keywords: Platelet concentrates, Fibrin, Centrifugation.

Introduction

These days the most common treatment alternative for an immature, permanent teeth that were earlier diagnosed with necrotic pulp were apexification with the help of calcium hydroxide (CH) or mineral trioxide aggregate (MTA). Most commonly, CH is potentially used as a root-canal dressing material besides the fact of observing the time-absorbing treatment which was found to potentially to increase the risk of fracture involving the root,[1] whereas apical MTA plugs proved to be much more effective when compared to the CH therapy, although the use of MTA is limited due to its expensive nature and difficult handling properties.[2] An effective alternatives that are available commercially are calcium silicate-based material and bio-dentine, which demonstrated quite some promising results, but its bonding with the composite material is greatly impaired.[3] However, the apexification procedure is highly independent of the material that was being used to create the apical barrier which showed the impossibility to allow for the revitalization and continued root development of the immature, necrotic tooth,[4] and hence compromising its prognosis eventually.[5,6]

Regenerative endodontics is an interesting and exciting field of newer treatment modality which has been used effectively for managing the necrotic teeth with an open apex. Murray *et al*.[7] elaborated about the regenerative endodontic procedures (REPs) as "biologically based protocol which was designed to replace the damaged and destructed tooth structures" such as root and dentin, along with the cells of the pulp-dentin complex. REP directly aims to pose a suitable environment (biomimetic microenvironment) in the root canal to enhance the repopulation of the canal with mesenchymal stem cells such as osteo/odontoprogenitor stem cells, regeneration of pulp tissue, and continued physiological root development.[8] Current REP protocol remains unable to reiterate the physiological structure and function and can probably induce the development of new vascularized tissue in the root-canal space. This guided endodontic repair process effectively allows for the continuing root development, thickening of root canal walls, apical closure, and further complete resolution of apical periodontitis.[9] The American Association of Endodontists postulated a regenerative endodontic protocol for the management of teeth with necrotic pulp and an immature apex that involves flooding the root canals with blood by frequent over-instrumentation of the canal.[10] An effective alternative in creating a blood clot is

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by the use of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), or other APCs.

APCs are the blood derivatives (from the patient's own blood) which contain few activated platelets that are being entangled within a scaffold of fibrin matrix. APCs were reportedly release few growth factors and cytokines which could play crucial roles in the regeneration process of teeth and its supporting structures that include cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis, and angiogenesis. Moreover, these processes were reported to promote healing of soft and hard tissues, APCs have been effectively used in the medical and dental fields over the past 10 years.[11] The classification of APCs postulated by the periodontology, oral surgery, esthetic and implant dentistry organization[12] involves the pure PRP (P-PRP), leukocyte- and PRP (L-PRP), pure PRF (P-PRF) or leukocyte-poor, PRF, and leukocyte- and PRF (L-PRF). PRP and PRF tend to form a three-dimensional fibrin matrix and are being used as REP scaffolds.[13] The composition of a blood clot was comprised 95% red blood cells (RBCs), 5% platelets, and <1% white blood cells, but the APCs (PRP and PRF) contain higher concentration of platelets which effectively incorporate various crucial growth factors, for example, platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-β), insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF), epidermal growth factor,and epithelial cell growth factor, within their agglomerated granules. Thus, both PRP and PRF may effectively prove to be a good alternative for the cell-based pulp/dentin regeneration.[14]

History of Platelet Derived Concentrates

The history of the platelet derived concentrates dated back to the 1970s, where the research works of Matras about the "Fibrin glues" were used to enhance the wound healing of skin in a rat model which is based on the concept that fibrin based matrix is the end result of the reaction chains of the coagulation and the first matrix of healing[15] and represented one of the first biological surgical adjuvants developed to enhance the process of healing.[16] Later on between 1975 and 1979, various new strategies were reiterated in formulating the effective role of platelets within the fibrin gel and upgraded the concept for the use of blood extracts which have been termed as "Platelet-fibrinogen-thrombin mixtures or platelets gels"[17] which were then applied in the various fields of oral and maxillofacial surgeries from 1997 onward.[18,19] Anitua further developed "Plasma rich in growth factors (PRGF)" by altering the procedures of PRP preparation.[20] All the earlier preparations, further, required extrinsic clotting factors to initiate the clotting pathways which were considered as the first generation of platelet concentrate.[21]

An another form of platelet concentrates which were proved to be self-clotted was developed by Choukroun *et al*. in France, in 2006 and named "PRF" due to the polymerization of the strong fibrin gel which was considered as the second-generation autologous concentrate.[22,23] Recently, authors have directed toward that these platelet concentrates were relatively associated with various forms of circulating cells particularly leukocytes which were relatively considered as a key element in the process of healing. Due to these reasons, the "L-PRF" was often briefed as an optimized blood clot[24] and allowed to brief the new therapeutic principle that is Natural Tissue Regeneration (NTR).[25]

Recently, various technologies and protocols were further developed to enhance the characterization of the preparations with respect to the release duration and content of growth factors or cells and physical properties which include "Advanced and Injectable PRF (A-PRF, i-PRF)."[12,26] The "Titanium-based PRF (T-PRF)", "Concentrated growth factors (CGF)," and "Autologous Fibrin Glue (AFG)" are various another modified form of PRF which is being prepared by changing the centrifugation protocol or material of collection tubes.[27,28] This long history initiates confusion in many which were related to the lack of specific terminology, characterization, and classification of the many products that were tested in the past four decades, and thus, their clinical relevance is largely debatable, as the literature on the topic is often confused and contradictory.[29]

Classification of Platelet Concentrates

The classification of the platelet concentrates has basically separated them based on two key parameters such as the presence of cell content (mostly leukocytes) and the fibrin imprints. This separation has enabled them elaborating into four major types of the products.[25]

- P-PRP or Leukocyte-Poor PRP: These formulations are without the presence of any leukocytes and with a low-density fibrin network after the activation so that they can be used as an injectable liquid solution or in an activated gel form to be effectively covered or injected on wounded or surgical site.
- L-PRP: These formulations are with leukocytes and with a low-density fibrin network and after the activation it can be made available as injectable liquid solutions or in an activated gel form which is to be placed or injected on wound or surgical site. Various technologies and commercial kits were equally developed to produce (L-PRP) with minimal handling of the blood samples and maximum standardization of the available preparations.
- P-PRF or Leukocyte-Poor PRF: These preparations are made without leukocytes and with a high-density fibrin network which can only exist in a strongly activated gel form so that it cannot be injected or used like traditional fibrin glues. Therefore, they can be effectively handled such as fibrin membranes or solid materials for many other applications.
- L-PRF: These preparations are made with leukocytes and with a high-density fibrin network and only exist in a strongly activated gel form and cannot be injected. However, due to their strong fibrin matrix, they can be handled like a real solid material for many applications. The technique was initially developed and evaluated as an

open-access technique, based on the concept of one-step centrifugation of blood without anticoagulant and without blood activator.[22]

Characteristics of Various Platelet Concentrates

PRP preparations

In this preparation, platelets are relatively small, irregularly shaped with a nuclear cells measuring 2–4 mm in diameter which is basically extracted from the fragmentation of the precursor megakaryocytes. The average life span of a platelet is ranged between 8 and 12 days. Platelets play a major role in the hemostasis and proved to be a natural source for the growth factors. Growth factors which are stored in the α-granules of platelets include PDGF, IGF, VEGF, and TGF-β. The release of growth factors is basically triggered by the activation of platelets which may be initiated by a variety of substances or stimuli such as thrombin, calcium chloride, collagen, or adenosine 5c-diphosphate. Moreover, to these growth factors, PRP comprises fibrinogen and a number of adhesive glycoproteins that help in cell migration. PRP could easily be prepared by two techniques. The techniques might vary in their technical aspects of preparation and are basically divided into:

- General purpose cell separators
- Platelet concentrating cell separators

General-purpose cell separators basically require some large quantities of blood (450 ml) and might require to be performed in a hospital-based setting. Blood is withdrawn into a collection bag which contains citrate-phosphate-dextrose anticoagulant. Initially, it is centrifuged at 5600 rpm to segregate RBCs from platelet-poor plasma (PPP) and PRP. The centrifugation speed is further reduced to 2400 rpm to attain a final separation of about 30 mL of PRP from the RBCs. Using this process, the remaining PPP and RBCs can be taken back to the patient's circulation or could eventually be discarded. The ELMD-500 (Medtronic Electromedic, Auto Transfusion System, Parker, CO, USA) cell separator is widely used for this technique.

Platelet concentrating cell separators are the most widely used equipment because of its portable nature which enabled them to be accommodated effectively in a dental clinic set-up. These technologies might allow the procurement of PRP by withdrawing smaller quantities of blood. Recently, two systems are effectively approved by FDA which was available commercially include, Harvest SmartPrep Platelet Concentrate System (Harvest Technologies, Plymouth, MA, USA) and the 3i Platelet Concentrate Collection System (3i PCCS; 3i Implant Innovations, Palm Beach Gardens, FL, USA).

Venous blood is allowed to be withdrawn into a tube containing an anticoagulant to stop platelet activation and degranulation. The first step is the effective centrifugation which is called "soft spin," that allows the separation of blood into three layers, namely, bottom-most RBC layer (55% of total volume), top most acellular plasma layer called PPP (40% of total volume), and an intermediate PRP layer (5% of total volume) called the "buffy coat." With the use of a sterile syringe, the operator then transfers PPP, PRP, and some RBCs into another tube without an anticoagulant. This tube is now subjected to undergo a second centrifugation which is relatively longer and faster than the first and hence called "hard spin" which, thereby, allows the platelets (PRP) to precipitate at the bottom of the tube with a very few RBCs left, which, thereby, effectively elaborates the red tinge of the final PRP preparation. The acellular plasma, PPP (80% of the volume), is reported at the top. Hence, the most of the PPP is removed with a syringe and discarded and the remaining PRP is shaken enough. Moreover, the resultant PRP is then mixed with bovine thrombin and calcium chloride at the time of its application. This effectively ended in gelling of the platelet concentrate. Calcium chloride effectively zeroes the effect of the anticoagulant that is citrate being used and thrombin assists in the activation of the fibrinogen which, further, gets converted to fibrin and cross-linked mass.[26]

Risks

The potential risks that were relatively associated with the usage of PRP are studied by Sanchez *et al*.[27] have detailed on the potential risks which are encountered with the use of PRP. The preparation of PRP involves the isolation of PRP after which gel formation is rapidly accelerated using calcium chloride and bovine thrombin. It has been observed that the use of bovine thrombin might be associated with the development of antibodies to the clotting factors V, XI, and thrombin which result in the risk of life-threatening coagulopathies which might develop. Bovine thrombin preparations have been effectively shown to contain factor V and resulted in the stimulation of the immune system when triggered by a foreign protein. Other methods followed effectively for a safer preparation of PRP include the utilization of recombinant human thrombin, autologous thrombin, or extra-purified thrombin. Landesberg *et al*.[28] have proposed that few alternative methods of activating PRP need to be studied effectively and made commercially available to the dental fraternity.

PRF preparations

In this type of preparations, Choukroun's P-PRF grouped to a new generation of platelet concentrates with a relatively simplified processing procedures and without any involved biochemicals in handling the blood. The study on PRF was initially led by Choukroun *et al*.[23,29] who proposed a standard protocol for the preparation of PRF which has to be followed to obtain a sufficient quantity and quality of the fibrin matrix, platelets, and growth factors; 24-gauge butterfly needle is effectively used for 9 mL blood collection in a sterile glass-coated plastic tubes without anticoagulant which are immediately centrifuged at 3000 rpm for about 10 min. During the process of centrifugation, if the blood comes in contact with the test-tube wall the platelet gets activated spontaneously leading to the initiation of coagulation cascade mechanism. After centrifugation, the resultant product comprises three layers basically. The top most layer consisting of acellular PPP, PRF clot in the middle, and RBCs at the bottom of the test tube. The fibrin clot resulted after centrifugation is removed from the tube and the attached RBCs were scraped off from it and discarded later. PRF can also be prepared in the

form of a membrane by squeezing out the fluids available in the fibrin clot.[30]

- L-PRF: The L-PRF clot or membrane are basically a modified PRF which contains mostly the platelets and leukocytes with the platelet growth factors and stem cells that are also entrapped within the fibrin network with increased strength.[31] It is created by the modification of initial technique of (P-PRF); blood should be collected relatively in 9 mL glass-coated plastic tubes (<20 s/tube) and immediately (before 1 min) which are allowed to be centrifuged in an intraspin centrifuge at room temperature (2700 rpm for 12 min) to produce L-PRF clots. The clots are then collected meticulously into a sterile adapted surgical box and were then compressed into membranes which remain as fibrin plug or then mixed with particulate bone for the preparation of sticky bone.[32]
- A-PRF: This is another modification from (P-PRF) in which it has been found that reducing the rpm while increasing the centrifuging time (1300 rpm, 14 min) resulted in an enhanced presence of neutrophilic granulocytes in the distal part of the clot with increased and prolonged release of growth factors. Therefore, this might able to influence the differentiation of host macrophages within the clot after implantation. Hence, they propose that this would influence the bone and soft-tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors.[33] However, other studies comparing A-PRF with L-PRF found contrast results regarding both growth factor releases and physical properties of the membrane.[12]
- i-PRF: The development of this injectable formulation of PRF was with the aim of delivering to clinicians an easy-to-use platelet concentrates in liquid formulation which can be either utilized alone or combined easily with various biomaterials. Taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed when compared to other formulations of PRF.[34] The i-PRF is produced as follows: 10 mL of whole blood collected in plain vacuum tubes without anticoagulant was immediately centrifuged at 700 rpm for 3 min. The 1 mL upper plasma layer was then collected using 21-gauge needle and designated as i-PRF.[20] Addition of i-PRF to particulate bone will lead to polymerization in 15 min to produce red colored sticky bone.
- T-PRF: Based on the fact that, titanium may be a potent platelet activator when compared to silica in preparing L-PRF. A study conducted by Tunalı *et al*. (2014) introduced a newer product called Titanium-prepared PRF in which the 9 mL of blood was quickly withdrawn in grade IV titanium tubes and the tubes were spontaneously centrifuged at 2800 rpm for 12 min and associated with the resultant which has more organized, thicker, and longer fibrin network.[35]
- CGFs preparation: This is an alternative centrifugation strategy which is used for this preparation (intravenous blood samples from the patients is placed in a standard, 10-ml non-anticoagulant centrifuge tubes and accelerated for 30 s, centrifuged at 2700 rpm for 4 min, 2400 rpm for 4 min, 2700 rpm for 4 min, and 3000 rpm for 3 min, and decelerated for 36 s to stop as adjusted automatically by centrifugal device) proposed that the separation of a fibrin matrix obtained is denser, larger, and enriched in growth factors; three layers were visually observed in the tube: RBC layer at the bottom, platelet-deprived plasma layer (without cell) at the top and fibrin gel with concentrated growth factor, and platelet aggregation in the middle. First, the uppermost portion which is deprived of platelet was then removed with a sterile syringe. This layer in the form of a membrane containing the concentrated growth membrane was held with the help of a hemostatic clamp which is separated from the RBC layer by separating with a pair of scissors and then pressed to form a membrane.[36]

AFG and sticky bone

This is a concept, wherein the fabrication of growth factors-enriched bone graft matrix (also known as "sticky bone") using AFG has been elucidated since 2010; to obtain an AFG, 20–60 cc of blood is collected in a non-coated tubes which are then centrifuged at 2400–2700 rpm for about 2 min. Out of the two layers that were obtained, the deeper layer resulted is RBC's, and the superficial layer is AFG. This AFG is, then, segregated using a syringe which is then mixed with particulate bone powder and was allowed to rest for 5–10 min for polymerization and therefore results in a yellow-colored mass called sticky bone.[37]

When comparing the biological effects of various platelet concentrates, PRP preparations were reported to enhance the process of osteogenesis by inducing and stimulating cells such as osteoblasts, which are derived from the local wounded site or from autologous graft tissue that were provided externally (osteoconductive). Furthermore, PRP in its gel form has also effectively provided a scaffold for all the incoming cells and growth factors and thus allowing the local physiological mechanism to take over but in an accelerated fashion. The apparent order of potency effect on the proliferation of human periosteal cells was PRP > CGF > A-PRF > PRGF while that of angiogenic potency is CGF/PRF > PRP/PRGF.[38] In addition to L-PRP's and PRF's direct effects on proliferation, differentiation, and angiogenesis, they also affect wound healing indirectly through the chemotactic recruitment of cells and local control of the inflammatory environment.[30,35]

The physical properties various different platelet concentrates could easily justify their possible clinical applications PRF and A-PRF membranes which could effectively serve as a resorbable membrane for GTR since they have a complex architecture of strong fibrin matrix with favorable mechanical properties which have the capability in preventing the migration of non-desirable cells into bone defect and by providing a space that allows the immigration of osteogenic and angiogenic cells, thus allowing the underlying blood clot

to mineralize spontaneously,[12] while CGF had larger and denser fibrin network contributed by thin and thick fibrin with multiple regenerative elements which were trapped among the fibrin network. The sticky bone forms strongly inter-linked fibrin network when mixed with the graft material that is mostly self-stable and thus the bone loss on the defect during the healing period is reduced without any use of bone tack or titanium mesh during or after the procedure.[29]

Clinical Outcomes in the Field of Regenerative Dentistry

To accelerate the process of tissue regeneration and repair in the field of medical and dental, platelet concentrates have been widely used.[39-41] With the use PRP preparations, a group of recent systematic reviews and meta-analysis suggested that there is no relative significant difference in bone augmentation in favor of the adjunctive use of PRP in bone formation, that is, osteogenesis around dental as well as other orthopedic implants[42] and there were no sufficient human studies that could strongly support the benefit of using PRP in the sinus augmentation procedures.[43] Therefore, for the effective management of infra-bony regenerative therapy, a systematic review and meta-analysis of 14 RCT's have recently published in the year 2018 and concluded that the combination of bone grafts with PRP was better producing acceptable clinical results in terms of CAL gain and pocket reduction than the adjunctive use of membranes after short- and long-term reassessment in-spite of the flap procedure and the high heterogeneity among the study participants.[44]

An earlier review conducted on the role of PRP in soft-tissue root-coverage procedures in 2008 had further concluded that the potential benefits of PRP in root-coverage procedures might be resulted improved esthetics, reduced patient morbidity, and accelerated spontaneous wound healing, while the effects of PRP in encouraging the root-coverage are not conclusive due to insufficient and lacking clinical evidence.[44] Later on, few clinical trials observed with the controversial results that the additional application of PRP with connective tissue graft shows significant increase in the width of the keratinized gingiva and gain in CAL,[45] while various other studies suggested that PRP can provide only an early healing of soft tissues with no clinically measurable improvements on the final therapeutic outcomes.[46,47] Regarding the use of APCs around implants and reconstructive surgery, the literature is directed dense with the use of the various forms of PRP – P-PRP or L-PRP. There are many plasma derivatives that had been effectively used during the implant placement (particularly as surface treatment for the stimulation of osseointegration) and in the management of peri-implant bone defects (after peri-implantitis, during implantation in an insufficient bone volume or during immediate post-extraction or post-avulsion re-implantation), sinus lift procedures and many other complex implant-supported therapies.

Fundamentally, for an effective management of the infra-bony defects, PRF used either in combination with bone grafts (bovine porous bone mineral, nanocrystalline hydroxyapatite, and demineralized freeze-dried bone allograft) or with other pharmacologic agents such as metformin gel which was found to be resulted in enhanced efficiency in terms of improvements in clinical parameters and depth reduction radiologically when compared to other bone grafts or when metformin used alone. Added to that, identical reduction in probing depth, gain in the clinical attachment level, and bone fill was seemed to be observed at sites which were treated with PRF or PRF with open flap debridement. Moreover, in the management of infra-bony defects, the use of PRF in furcation defects when combined with bone grafts (hydroxyapatite) and rosuvastatin has demonstrated better results in iterating its role in periodontal regeneration.[41] PRF has also shown remarkable positive healing effects when used for the preservation of extraction socket and as a sole grafting material in sinus lift procedures during simultaneous dental implantation.[48]

Future Perspective

Hence, the following criteria need to be focused in the near future,

a) The study of the sustained or on demand delivery of growth factors present in platelet concentrates.
b) Characterization of PRP and PRF proteomes is highly recommended to enable more robust and predictable clinical outcomes.
c) Further research to be done on the efficacy of APCs on stem cell behavior, proliferation, and differentiation which are to be done for cell-based therapies.
d) Further *in vivo* studies and clinical trials to be carried out.
e) We need more data to opt out the calculated therapeutic doses for platelet concentrates which are suitable for various clinical therapies.
f) Future clinical trials are now further directed to investigate the potentiality of utilizing platelet concentrates for soft-tissue regenerative strategies in combination with various biomaterials, pharmaceutical agents, or stem cells.

Conclusion

PRP is considered as a new therapeutic option for different treatment modalities in the field of dermatology and cosmetology. Hence, understanding the biology and mechanism of action of PRP therapy will help the clinicians in selecting specific system/devices and characterizing the type of PRP which helps in standardization of PRP, making it less easy to sort and interpret the available findings from various studies. Since there is no double blind, randomized, placebo-controlled trials conducted on a large sample size to constitute a good quality of evidence. Thus, a healthy amount of caution should be exercised by the treating physician with appropriate preparation and use during PRP procedures.

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