Review Article

PLATELET CONCENTRATES FOR PERIODONTAL REGENERATION

ANNALS OF DENTISTRY
UNIVERSITY OF MALAYA
www.adum.edu.my

Ann Dent UM 2020, 27:55-65
DoI: 10.22452/adum.vol27no9

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KEYWORDS

Platelet concentrates, platelet rich plasma, platelet rich fibrin, injectable platelet-rich fibrin, titanium platelet-rich fibrin.

ABSTRACT

Platelet concentrates are derivatives of blood that aid in haemostasis and wound healing after periodontal regenerative procedures. Its ability to act as a natural scaffold of growth factors has gained significance in many surgical procedures. This narrative review discusses the different platelet concentrates, their centrifugation protocols, advantages and disadvantages and their application in periodontal regenerative procedures. An electronic search of PubMed or MEDLINE was conducted for relevant material from the published literature up to 2020. The key words looked for were "Platelet concentrates, Platelet rich plasma, Platelet rich fibrin and periodontal regeneration." We have used the filters comparative human studies, animal studies, randomized controlled trials, case reports and systematic reviews. The searches were limited to articles in English language and articles describing platelet concentrates and its relation to periodontal regeneration were collected and used to prepare a concise review.

INTRODUCTION

Periodontitis is an inflammatory disease of the periodontium that leads to loss of tooth supporting tissues. Following periodontal therapy, healing occurs by regeneration, repair or a combination of both. The healing process depends on the availability of cell types needed and signalling cascades that regulate stimulation of these cells. The natural wound healing cascade is commenced by clot formation accompanied by proliferative and maturation phases. Thus, there is fibrin formation, platelet aggregation and release of several growth factors into tissues from platelets. Growth factors also aid in wound healing by favouring mitogenesis, chemotaxis, and angiogenesis [1,2].

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Platelets are small irregularly shaped cells present in blood that are derived from precursor megakaryocytes. They are approximately 2–3 µm in diameter, and constitute granules, few mitochondria, and prominent membrane structures. The canalicular system and a well-stacked tubular system on the cell surface helps in expulsion of growth factors upon platelet activation [3]. The substances located in the α-granules, dense granules, and lysosomes of platelets modulate its activation. Of which, the most abundant ones are α-granules that contains many bioactive mediators. During tissue injury, the platelets get activated and release wound healing factors like platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and epidermal growth factor (EGF) [4,5]. As the direct platelet influence subsides, macrophages arrive by means of vascular ingrowth stimulated by the platelets and assumes responsibility for wound-healing regulation by secreting their own factors. Thus, the platelets set the pace for wound repair [5].

As platelets contain biologically active proteins, they create a chemotactic gradient for recruitment

of stem cells which undergoes differentiation and may promote healing by regeneration. Hence, autologous platelet concentrates have a promising scope in periodontal regeneration. In this review, we aimed to briefly describe the different platelet concentrates and their application in periodontal regenerative therapy.

HISTORY OF PLATELET CONCENTRATES

The first platelet product used as a surgical adjuvant was the "Fibrin glue" by Matras in 1970, that improved skin wound healing in rat models [6]. The regenerative potential of platelets was initially introduced in 1974 by Ross et al [7] and in 1986, Knighton et al [8], termed platelet concentrates as "platelet-derived wound healing factors" as they promoted healing when used for skin ulcers. In 1998, Marx et al [9] introduced the first generation of platelet concentrates known as platelet rich plasma (PRP) and in 2000, Choukroun et al [10] introduced the "second-generation" of platelet concentrate known as platelet rich fibrin (PRF). Bielecki et al [11] and Cieslik-Bielecka et al [12,13] defined PRP as an inactive substance and Platelet Rich Gel (PRG) as a biologically activated fibrin matrix in 2006. The concept of concentrated growth factors (CGF) was introduced by Sacco in 2006 which were found to be rich and dense fibrin blocks [14]. The first classification on platelet concentrates was proposed by Dohan Ehrenfest et al [15] in 2009 based on 2 key parameters that is, presence of cell content (mostly leukocytes) and the fibrin architecture. This classification included 4 main families of products: Pure platelet-rich plasma (P-PRP) or leukocyte-poor platelet rich plasma, Leukocyte and platelet-rich plasma (L-PRP), Pure PRF (P-PRF) or leukocyte-poor PRF and Leukocyte and platelet-rich fibrin (L-PRF) (Figure 1). Subsequently Sohn introduced the concept of sticky bone in 2010 [16]. Recently, certain modifications of platelet rich fibrin were introduced. These were the advanced platelet rich fibrin (A-PRF) introduced by Choukroun in 2014 [17], Titanium prepared platelet rich fibrin (T-PRF) by Tunali et al [18] and injectable PRF (i-PRF) by Mourão et al in 2015 [19].

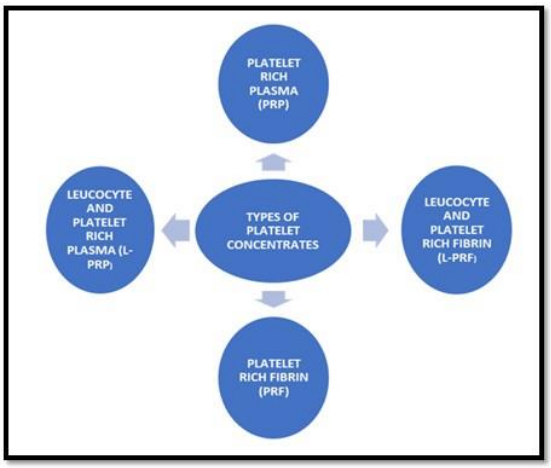

Figure 1: Types of Platelet Concentrates

DIFFERENT TYPES OF PLATELET CONCENTRATES AND ITS CLINICAL APPLICATION

PLATELET-RICH PLASMA (PRP)

PRP mimics the terminal stage of coagulation cascade, that is fibrin clot formation. The release of growth factors through α granules increases early wound strength by cell proliferation and angiogenesis [20]. It also eliminates the risk of disease transmission due to its procurement from autologous blood.

Most commonly used method for preparation of PRP is Curasan method [21]. After drawing blood from a suitable patient, it undergoes a two-step centrifugation process. The first spin separates the RBC's from plasma after running for 10 mins at 2400 revolutions per minute (rpm). Then platelet poor plasma and the PRP with its "buffy coat" is transferred into a separate tube and then centrifugated for 15 min at 3600 rpm (Figure *2).* This gives pure PRP. The resultant product consists of 3 layers: A top layer of PPP; a middle layer, also called "buffy coat" of white blood cells and platelets; and a bottom layer of red blood cells. PRP is the middle layer. Then the PRP that is obtained is mixed with a sterile solution containing 10% calcium chloride and 100U/ mL of bovine thrombin and a gel is formed [21].

Clinical applications

a. Sinus Lift Procedures.

b. Ridge Augmentations.

c. Socket Preservation.

d. Oro-nasal fistula repair.

e. Jaw reconstruction surgeries.

f. Intra Bony Defects.

g. Soft Tissue Procedures.

Various animal experiments, human clinical and randomized controlled trials, systematic reviews and meta-analysis have been conducted to analyse the efficacy of PRP in various periodontal regenerative procedures.

Animal experiments

Suaid et al conducted a histometric study in dogs and reported that when a combination of PRP with subepithelial connective tissue graft was used for treatment of gingival recessions, it was very effective in promoting formation of new cementum than when the graft was used alone [22].

Marcelo Diniz Carvalho et al reported that PRP failed to exert a significant effect on periodontal regeneration when combined with bovine glass for treatment of three walled intrabony defects in dogs [23].

Clinical trials

In a study conducted by Anitua, it was reported that there was an improvement in soft tissue repair and bone regeneration inside the extraction socket after applying PRP and that these sites could be used for implant placement in future [24].

Lekovic et al conducted a study in humans to compare a combination of bovine porous bone mineral, PRP, and guided tissue regeneration for management of intrabony defects. The study concluded that PRP had strong regenerative potential resulting in reduction of pocket depth and gain in clinical attachment level [25].

Wiltfang et al reported that PRP enhanced bony regeneration but did not affect the degradation of tricalcium phosphate material when beta-tricalcium phosphate was combined with PRP for sinus floor augmentation [26].

Riaz et al analysed the efficacy of PRP with hydroxyapatite crystals for maxillary sinus augmentation and reported that it was very effective in increasing the height of residual alveolar bone [27]. Dutta et al reported that autologous PRP was clinically biocompatible and showed improvement in soft tissue healing, bone regeneration and increased bone density in extraction sockets of mandibular third molar [28].

Srinivas et al reported that there was a significant improvement in clinical parameters when subepithelial connective tissue graft was used along with PRP to treat miller's class I and II gingival recession [29].

Randomized controlled clinical trials

A randomized controlled clinical trial reported that PRP improved the osteoconductive properties of anorganic bovine bone and increased bone regeneration in sinus floor augmentation [30].

Kutkut et al conducted a single-site, randomized controlled investigation to evaluate clinical and histologic outcome of using medical-grade calcium sulfate hemihydrate mixed with PRP for extraction socket preservation before implant placement. It was shown to regenerate greater percentage of new vital bone at 3 months with enhanced healing of bone [31].

In a randomized controlled clinical trial conducted by Eskan et al, PRP was found to enhance bone regeneration when combined with a cancellous allograft for ridge augmentation [32].

Systematic review and meta-analysis

Panda et al systematically evaluated the clinical and radiological outcomes of the additive efficacy of autologous platelet concentrates in treatment of intrabony defects when used alone and along with other regenerative procedures. PRP was found to be effective when used as an adjunct to grafting materials, but ineffective when used in combination with guided tissue regeneration procedures [33].

Advantages:

a. PRP enhances rapid regeneration by bringing cytokines and growth factors to the site.

b. It is free from concerns over transmissible disease.

c. Also, it is convenient and economical for patient.

Disadvantages:

a. Preparation protocol of PRP lacks standardization.

b. Although never reported, the addition of bovine thrombin to the platelet concentrate could cause adverse reactions such as systemic lupus erythematosus.

These disadvantages have reduced the usage of PRP [34] and led to evolution of "second generation PRP" coined as Platelet rich fibrin which is purely an autologous human thrombin [20].

PLATELET RICH FIBRIN (PRF)

The PRF clot is obtained by the natural polymerization process during centrifugation. The fibrin architecture has a distinctive property of slow release of important growth factors such as transforming growth factor β, platelet-derived growth factor, vascular endothelial growth factor and matrix glycoproteins such as thrombospondin-1 and cytokines for a period of about 28 days [35]. This enhances strong reparative and regenerative processes [36,37]. It also forms a trimolecular fibrin meshwork making it flexible to support cytokine function and cellular migration [34,37].

The ideal technique for PRF preparation was first put forward by Choukroun et al [10]. It involves collection of 5-ml of venous blood in two separate 6-ml tubes that are not coated with an anticoagulant and centrifuged at 3000 rpm at 400 g for a period of 12 mins (Figure 2) [10].

The end product consists of 3 layers: cellular PPP as the topmost layer, middle layer of PRF clot and bottom layer of red blood cells [37].

The main setback of this technique involves rapid coagulation of blood initiated on contact with the wall of the test tube. So, it is important to speed up the centrifugation process giving less working time for the clinician. It can also be moulded as a membrane by squeezing out fluids present in the fibrin clot [38,39].

Clinical applications

a. Inclusion with Bone Grafts to accelerate the healing process.

b. Socket Preservation.

c. Root Coverage.

d. Intra Bony Defects and Furcation Defects.

e. As free Gingival Grafts for healing of palatal wound.

f. In sinus lift procedures.

Currently many studies have highlighted the regenerative potential of PRF when used in various periodontal surgical procedures and they are briefly described below.

Animal studies

Duan et al suggested that PRF combined with rat periodontal ligament stem cells provided a valuable tool for periodontal tissue engineering [40].

An animal study by Wang et al reported that the combined use of lyophilized PRF and osteogenic bone marrow mesenchymal stem cell sheet fragments enabled bone tissue regeneration. They suggested that the use of this combination may provide insights into the fabrication of engineered bone [41] .

Clinical trials

A study by Deepa and Jain (2015) has shown enhanced healing of gingival fenestrations after the use of PRF which suggested its use as a membrane in aesthetically demanding areas [42].

Al-Maawi et al studied on the interaction between 5 different collagen membranes like Mucograft, Bio-Gide, Mucoderm. Collprotect and BEGO with liquid PRF. It was concluded that it was possible to load these membranes with PRF which might help to enhance its bioactivity [43].

Randomized controlled clinical trial

Pradeep et al observed an improvement in clinical parameters when PRF was used in the regeneration of intra bony and furcation defects [44].

Agarwal et al evaluated the effect of PRF in combination with a decalcified freeze-dried bone allograft for treatment of intrabony defects and noted significant improvement in clinical and radiographic parameters after 12months [45].

It was suggested the use of PRF in combination with demineralized freeze-dried bone allograft (DFDBA) for socket preservation as they observed a significantly lower gain in alveolar ridge dimensions with the use of DFDBA alone [46]. Another authors suggested that use of PRF membranes in multiple layers effectively increased the thickness of keratinized tissue when used for the treatment of Miller's Class I gingival recession [47].

Atchuta et al reported that a combination of PRF and DFDBA improved the clinical and radiographic parameters when used in regeneration of intrabony defects in chronic periodontitis patients [48].

Systematic review and meta-analysis

Based on their systematic review, Strauss et al concluded that the use of PRF would increase implant stability by reducing alveolar bone resorption and also would reduce postoperative pain and enhance wound healing after implant therapy [49].

A meta-analysis by Li et al proved that the synergistic application of PRF and 1% alendronate provided better results during periodontal bone regeneration [50].

Rodas et al reported in his systematic review and meta-analysis that PRF membranes are a promising alternative to autogenous gingival grafts in the treatment of Miller class I and II gingival recessions [51].

Advantages of PRF over PRP:

a. Ease of preparation and application,

b. Cost effective.

c. Reduced incidence of adverse reactions due to absence of bovine thrombin or anticoagulants.

Disadvantages:

a. The relatively low quantity of PRF obtained from samples.

b. PRF should be immediately used after preparation as it can lose the structural integrity by shrinkage due to dehydration [52].

LEUCOCYTE PLATELET RICH FIBRIN (L-PRF)

These are modified PRF clot or membrane that contains most of the platelets and leukocytes from the initial blood harvest plus platelet growth factors and stem cells entrapped within the fibrin network with enhanced strength [53]. It is obtained by modification of technique of pure platelet rich fibrin. Blood should be collected in less than 20 seconds in 9ml glass-coated plastic tubes. It is then immediately centrifuged at 2700 rpm for 12 minutes to produce L-PRF clots (Figure 2). The clots are then collected into a sterile surgical box and are compressed into membranes. It could be used as a fibrin plug. It can also be used for sticky bone preparation by mixing with particulate bone.[54] A randomized controlled pilot clinical trial by Temmerman A et al has shown promising results when L-PRF membrane was used for increasing the width of keratinized mucosa around implants [55].

ADVANCED PLATELET-RICH FIBRIN (A-PRF)

A-PRF is another modified form of pure platelet rich fibrin obtained by decreasing the rpm and increasing the centrifugation time that is 1300 rpm for 14 minutes (Figure 2). The resultant clot has an increased number of neutrophils and macrophages [53]. It is truly a revolutionary concept due to its ease of handling and the fact that it can be modified easily in a relatively short period of time. Also it provides the defect not only with a matrix that permits cell migration into the defected area but also provides important biological factors that accelerates wound-healing such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), platelet factor 4 (PF4), IL-1, vascular endothelial growth factor (VEGF), epidermal growth factor, endothelial cell growth factor (ECGF), platelet-derived endothelial growth factor (PDEGF), insulin-like growth factor, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin [56]. Since it is a matrix that is obtained from patient's own blood, it eliminates the risk of foreign body reaction. It contributes to wound healing, tissue repair and can be used for tissue regeneration [57]. An invitro study revealed that both L-PRF and A-PRF stimulated the proliferation of periodontal ligament fibroblasts. A-PRF showed enhanced wound healing ability than L-PRF [58]. Positive results have been shown when A-PRF was used for treatment of gingival recessions [59,60].

INJECTABLE PLATELET-RICH FIBRIN (I-PRF)

Injectable form of PRF is a platelet concentrate in liquid formulation which can be used alone or in combination with other biomaterials. It was observed to have a higher presence of regenerative cells and growth factors as compared to other forms of PRF. It also clots and attains a gel form after about 10-15 minutes for sustained release of growth factors in the tissue and induces expression of transforming growth factor-β and collagen-1 mRNA [61].

I-PRF was produced by collecting blood in a 9 mL tube without anticoagulants and centrifuging it for 2 mins at 3300 rpm (Figure 2). The resultant

product obtained in the tube is an orange coloured fluid called I-PRF [19].

Recently a randomized controlled clinical trial observed that the application of i-PRF by a non-surgical method influenced the increase in gingival thickness in individuals with thin periodontal phenotypes [62].

TITANIUM PLATELET- RICH FIBRIN (T-PRF)

T-PRF was introduced by Tunali et al in 2014, based on the hypothesis that titanium may be more efficient in activating platelets than silica used within glass tubes which were conventionally used for preparation of other platelet rich fibrin concentrates [18]. Although the use of L-PRF in regenerative procedures has been found to be successful, the use of glass tubes for blood collection has raised an issue of the possible health hazard due to the unavoidable silica contact as reported by O' Connell. These silica particles were found to remain colloidally suspended in the 3 layers of the end product obtained after centrifugation thus posing a risk of these particles reaching the patient when used for treatment [63].

T-PRF is prepared by collecting 9ml blood drawn from the antecubital vein into Grade IV titanium tubes and immediately centrifuging at 2800 rpm for 12 mins (Figure 2) [64]. The study conducted by Tunali et al found that the fibrin of T-PRF seemed to be more tightly woven and thicker than L-PRF [65]. As a result, it would remain in the tissue for a longer time [66].

Recently several randomized controlled clinical trials were conducted to evaluate the regenerative potential of T-PRF.

In a study by Chatterjee et al, use of T-PRF in the treatment of intra bony defects demonstrated significant improvements in periodontal parameters and radiographic findings [67].

An improvement in periodontal healing was observed when T-PRF membrane was combined with open flap debridement. This platelet concentrate also provided significantly higher concentrations of growth factors and lower RANKL/OPG ratio in GCF for about 4 to 6 weeks [68].

Another study that compared T-PRF and GTR when combined with open flap debridement showed significant improvement in clinical parameters compared with open flap debridement alone in treatment of intra bony defects with endo-perio lesions. The authors concluded that the use of T-PRF may give similar successful results as GTR [69].

CONCENTRATED GROWTH FACTORS PREPARATION (CGF)

The preparation protocol used for CGF yields a much denser and larger fibrin matrix rich in growth factors. Intravenous blood samples are drawn from the patient into 10-ml non-anticoagulant centrifuge tubes. It is then 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 then decelerated for 36 s to stop (Figure 2). Three layers are observed in the tube: a bottom layer red blood cells, a middle layer of fibrin gel with concentrated growth factor and platelet aggregation and a top layer of platelet-deprived plasma. First, the uppermost platelet-deprived fraction is removed with a sterile syringe. Then the layer containing concentrated growth factor is separated from the other two layers and pressed into a membrane [70].

It acts as a fibrin tissue adhesive thus acting as an effective haemostatic agent. It helps in wound healing and accelerates osteogenesis. It enhances attachment of a new connective tissue to the root surface thus improving wound stability. It also acts as a scaffold that helps in attachment of cytokines and cellular migration. CGF promotes epithelial, endothelial and epidermal regeneration and decreases dermal scarring. It possesses an antimicrobial as well as an anti-angiogenic property on chronic non healing wounds [71].

Clinical trials have shown that CGFs can act as an alternative to bone grafting and could be used for bone regeneration for sinus augmentation [72] and as a membrane support in implants to accelerate bone integration [73].

Results of a randomized clinical trial suggested that the use of CGF in combination with coronally advanced flap may increase the success of gingival recession treatment as there was an increase in keratinized gingival width and gingival thickness [74].

CGF when applied with bone graft materials could be used in treatment of bone defects which is based on significant radiographic defect bone fill. It also acts as an effective barrier to regenerate bone when associated with GBR and GTR procedures [75].

AUTOLOGOUS FIBRIN GLUE (AFG) AND STICKY BONE

The concept of mixing autologous fibrin glue with bone graft to obtain sticky bone was introduced by Sohn in the 2010 [76]. Autologous fibrin glue was obtained by centrifuging 20 - 60CC of blood in non-coated tubes at 2400-2700 rpm for 2 mins to obtain two layers. The RBC's form the bottom layer and autologous fibrin glue forms the superficial layer. Then AFG was extracted using a syringe and mixed with particulate bone powder. It was allowed to rest for 5-10 mins for polymerization and this resulted in a yellow coloured mass called sticky bone [73]. It can used for space maintenance, angiogenesis and tension free primary suture in guided bone regeneration [77,78].

It was found in a study that application of autologous fibrin glue after free gingival graft operation in the palatal donor region reduced post-operative complications and accelerated wound healing [79].

The use of "sticky bone" preparation was found to be useful for alveolar ridge augmentation as bone graft trapped within cross-linked fibrin meshwork prevented any undesirable movement of graft particles during the healing phase. This stabilized the bone graft onto the defect without the need of using any bone tacks or titanium mesh and this promoted tissue healing. The fibrin interconnection prevents ingrowth of soft tissue into the sticky bone graft [73].

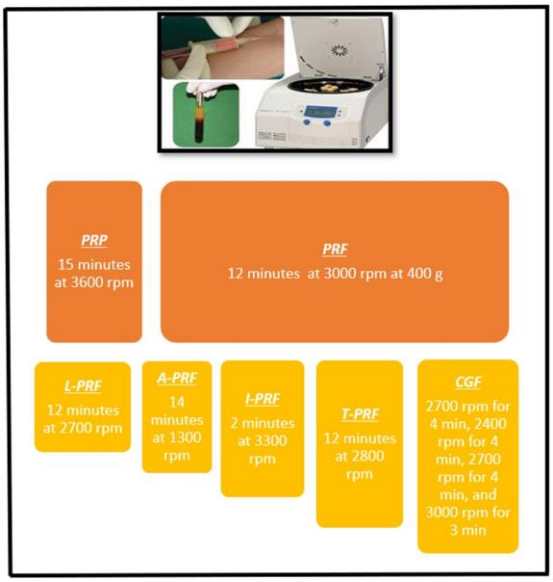

Figure 2: Centrifugation Protocol for Platelet Concentrates

FUTURE PERSPECTIVES

Platelets are being proposed to be used as carriers for loading drugs or biological therapies to specific target locations. Studies conducted on platelets have shown their capability of recognizing and interacting with tumour cells. So, development of new drug delivery systems and therapeutic strategies could be of use in management of tumours as this might help to minimize the side effects of chemotherapy such as cytotoxicity and non-specific targeting [80].

In a study that was conducted recently in a mouse model of lymphoma, doxorubicin loaded platelets enhanced intracellular accumulation of drugs in tumour cells through "tumour cell-induced platelet aggregation". This improved the anti-tumour activity of doxorubicin [81]. Zhen Gu and collaborators generated PD-1-expressing platelets to use it as post-surgery consolidation treatment in tumours. This could accumulate within the tumour surgical wound enhancing its anti-tumour immune response thus eliminating the residual tumour cells which could result in relapse of the tumour locally and distally [82].

One of the most abundant cell-derived microparticles are Platelet-derived microparticles (PMPs or platelet "dust") which are produced by platelets upon activation [83]. Various studies on these microparticles suggests its role as modulators of immune system as well as its role in various diseases [84]. PMPs also play a potential role in thrombosis and haemostasis, so a defect in the production of PMPs is associated with bleeding complications as seen in Scott syndrome patients [85,86]. These microparticles have gained attention as potential diagnostic markers when used for detection of rheumatoid arthritis and cardiovascular diseases [87,88].

The use of advanced delivery systems such as liposomes for encapsulating the platelet concentrates has proved advantageous due to its biocompatibility, low immunogenicity, protection of growth factors against enzymatic degradation, and long-term bioavailability as well as ease of surface modification for selective targeted delivery. In vitro studies have shown favourable results for enhanced bone regeneration when biodegradable scaffolds such as calcium phosphates and poly lactic-co-glycolic acid were combined with biopolymers such as hyaluronic acid and gelatin for encapsulating PRP [89].

CONCLUSION

The use of platelet concentrates is a novel approach in the field of periodontal regenerative procedures. This is due to their ability to harbour growth factors that enables enhanced wound healing and accelerated regeneration of periodontal tissues. Being a natural and economical autologous product, it eliminates the risk of adverse reactions and disease transmission. Although various studies based on treatments using platelet concentrates have provided appropriate outcomes, further studies that includes large populations are still required to establish its efficacy.

ACKNOWLEDGEMENT

The authors would like to acknowledge the authors and publishers of all those articles and journals that has been cited in this manuscript.

DECLARATION OF INTEREST

The author reports no conflicts of interest. The author alone is responsible with the contents of the article.

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Editorial History

Date of Submission: 12 September 2020

Review and Revision: 15 October 2020 to 13 November 2020

Accepted: 22 November 2020

Published: 01 December 2020