



## **Application of Platelet Rich Fibrin, Platelet Rich Plasma, Plasma Rich Growth Factors in Different Fields of Dentistry: An Overview**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author GS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BK and AG managed the analyses of author HSG, AM and FJ managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

A huge number of studies have demonstrated that platelet-rich preparations applied to surgical sites, injuries, or wounds are effective way to promote soft tissue healing and bone growth. The potential use of platelet rich preparations like Platelet - rich plasma and Platelet - rich fibrin are new boon to dentistry termed under the general acronyms PRP (Platelet-Rich Plasma) or PRF (Platelet-Rich Fibrin), is an important current transversal field of research across many fundamental and clinical disciplines. The third important is PRGF. Plasma Rich in Growth Factor

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(or PRGF) is a type of plasma enriched of proteins and circulating growth factors able to aid the bone and soft tissue regeneration. PRGF includes plasma proteins and coagulative factors and is then more valuable compared to PRP. There are many scientific literature which cover one or other concise aspect of platelet rich preparation. This review aims at covering all basic definitions, key element, history, preparation, differences between PRF and PRP and applications in a simplified manner.

**Keywords:** *Platelet rich plasma; platelet rich fibrin; plasma rich in growth factor; graft material; wound healing; regenerative method.*

## 1. INTRODUCTION

As per ongoing logical examinations, it has been indicated that platelets assume a significant part in wound recuperating and tissue fix and vascular rebuilding other than incendiary and safe reaction. Platelets contain organically dynamic proteins, authoritative of these proteins inside a creating fibrin work or to extracellular network can make chemotactic slopes preferring enlistment of foundational microorganisms, invigorating cell movement, separation, and advancing repair [1,2]. The key components needed to advance tissue mending and recovery are: the fibrin (filling in as a supporting grid), the platelets (wealthy in development variables), and cells (generally the different populaces of leukocytes, and undifferentiated organisms for their antibacterial, neovascularization and regenerative properties) [3]. The improvement of platelet concentrates for careful use, regularly named under the overall abbreviations PRP (Platelet-Rich Plasma) or PRF (Platelet-Rich Fibrin), is a significant flow cross-over field of examination across numerous essential and clinical disciplines [4]. Platelet Rich Plasma is original concentrate with short cytokine discharge and furthermore poor mechanical property which lead to the chase of better focuses. Most recent improvement lead to the subsequent age concentrate called PRF (Platelet Rich Fibrin) which improved mechanical properties, less work and is likewise cheap when contrasted with PRP. Consequently, PRF(Platelet Rich Fibrin) turned out to be more well known in dentistry with time [2]. There is a ton of disarray between the these three packs and in numerous written works considered same which is debatable [4]. Plasma wealthy in development factors (PRGF) has more extensive use in numerous fields of dentistry because of its endogenous biocompatible regenerative potential [48]. PRGF builds epithelial to mesenchymal change, and wipes out epithelial homes in the hidden connective tissue, along these lines it adds to the rebuilding and capacity of unique tissues. Platelets go about as transporters of endogenous

morphogens that may balance cell destiny and hence influence tissue structure and capacity. PRGF may tweak the quality articulation of numerous cells, for example, chondrocytes, synoviocytes, macrophages, mesenchymal immature microorganisms, and subsequently impact an anabolic microenvironment and diminish torment and improve the tissue functioning. Hence, this article targets streamlining the current distinction between the three concentrates and furthermore gives a survey to the historical backdrop of advancement and utilization of PRF, PRGF and PRP in dentistry.

## 2. DEFINITION

### 2.1 What is PRP?

**Definition:** PRP is a biological product defined as a portion of the plasma fraction of autologous blood with a platelet concentration above the baseline (before centrifugation) [5]

Otherwise called Platelet rich development factor, PRF grid and Platelet concentrates [6]. The idea of PRP began in the field of hematology [7]. Hematologist made the term PRP during the 1970s so as to depict the plasma with platelet tally over that of fringe blood, which was at first utilized as a bonding item to treat patients with thrombocytopenia [8]. The standard platelet concentrate for bonding has been named PRP and traditionally contains  $0.5 \times 10^{11}$  platelets for every unit [9]. Although PRP was not effective because of its moderate cytokine, helpless versatility and cost. It was very mainstream in 1970s after 10 years PRF was utilized in maxillofacial medical procedure as fibrin had the potential for adherence and homeostatic properties. This new item resembled an autologous cyatrical matrix [10].

### 2.2 What is PRF?

**Definition:** PRF is an autologous fibrin based (membrane, matrix or scaffolded), living

biomaterial derived from human blood clot [11,12,13,14,15] also referred as optimized blood clot [16]. PRF is also known as Second generation concentrate. PRF (Platelet Rich Fibrin) was first used in 2001 by Choukroun, et al. in oral and maxillofacial surgery [17]. It was developed and termed in France [4]. During the centrifuge process the blood coagulate and is separated in three distinct layers (Fig. 1) upper straw colored which is acellular plasma, middle which contains fibrin clot and lower portion consist of Red colored RBC [17]. The upper and bottom layer is discarded, middle layer is fibrin mesh contains platelet. The middle layer is whitish in color and known as Buffy coat and can be observed by naked eye [3]. The PRF can be used directly as clot or after compression as membrane. The key element in PRF (Fig. 2) to promote healing are Fibrin which consist of supporting matrix, Platelets which are rich in growth factor and Cells like leukocytes and stem cells are also present. These cells attribute to unique properties like antibacterial, neovascularization and regeneration properties which promote healing [14]. The leukocyte and platelet rich fibrin (L-PRF) is often called “optimized blood clot” that can be surgically handled and used [14].

### 2.3 What is PRGF?

Plasma Rich in Growth Factor (or PRGF) is a kind of plasma advanced of proteins and circulating development factors ready to help the bone and delicate tissue recovery. PRGF readiness was done by following a formerly portrayed convention. Blood test was gotten from basil-ic vein utilizing an enormous needle to evade platelet rup-ture. Inspected blood was joined with antico-agulant (1 ml of 3.8% sodium citrate for 10 ml blood) and centrifuged at 460 G quickly: af-ter the centrifugation, PRGF was taken from the lower part of the cylinder. Calcium Chloride was then added to PRGF (0.05 ml per ml): this activity favorable to bits the coagulation, normally acquired inside all things considered 10 minutes. At the finish of the system, we got a thick PRGF, to be quickly positioned in the careful site [18].

### 3. HISTORY

History of these strategy began a lot before with the exploration work of Matras in 1970s about the fibrin pastes used to improve skin twisted mending in rodent model [19] Following the standard created with fibrin sticks a couple of

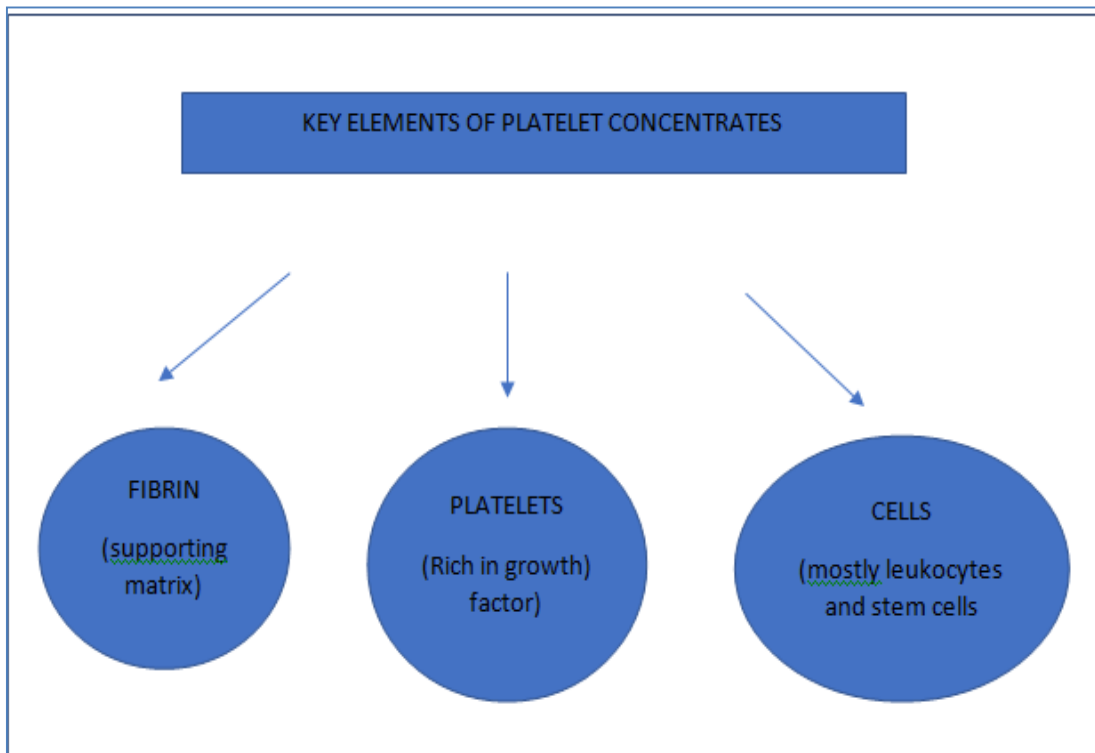
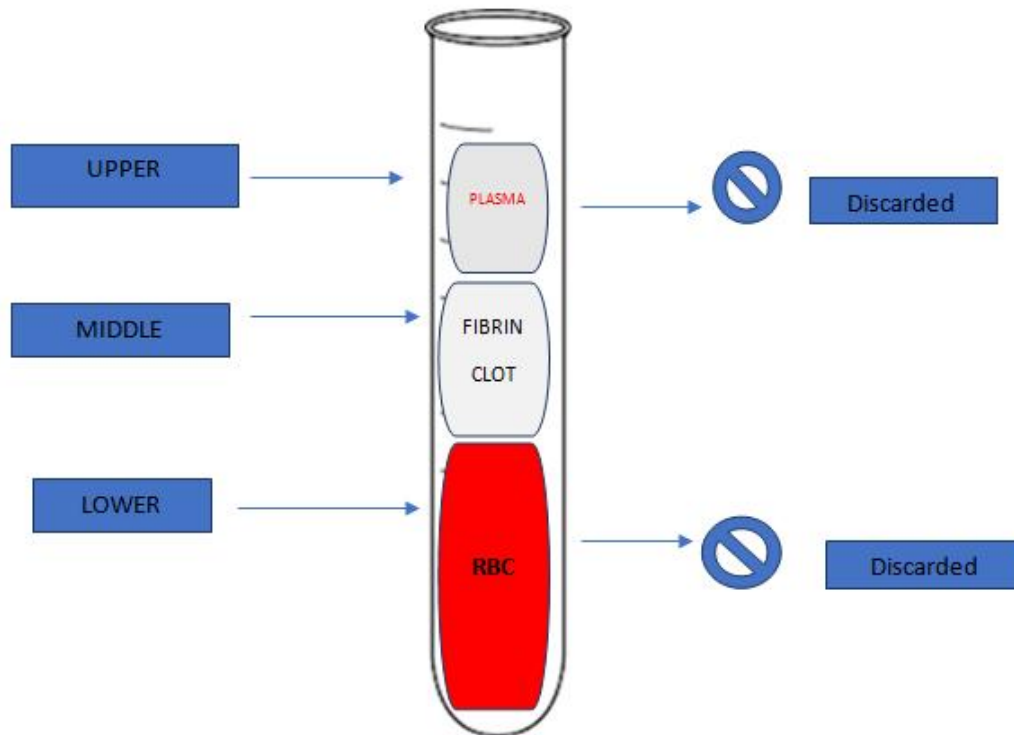


Fig. 1. Key elements of platelet concentrates



**Fig. 2. Plasma analysis techniques**

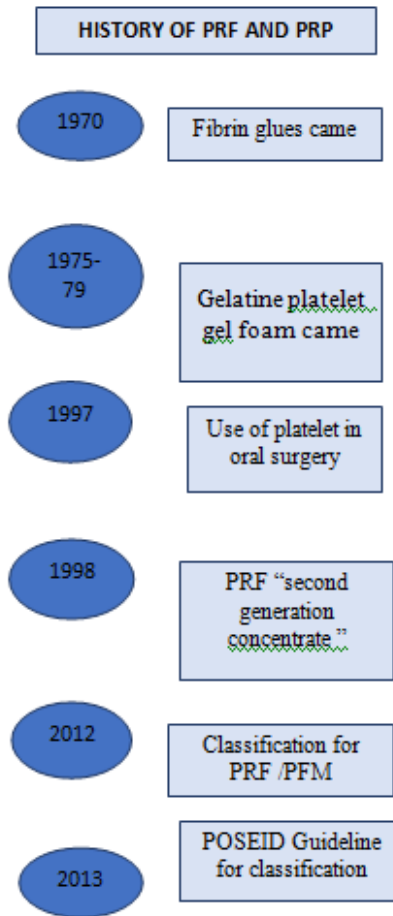
year later (1975-79) (Fig. 3) an overhaul idea for the utilization of blood separate sprung up. It was designated "Platelet-Fibrinogen-Thrombin-blend" or gelatine platelet gel foam [4]. These strategies kept on growing gradually until the article of Whitman in 199719 and Marx et al in 199820 came. These articles are celebrated for its commitment in the field of Oral and maxillofacial medical procedure and regenerative medication. All aggregates around then was called PRP later wordings were given when another type of platelet moves was created in France and named Platelet-Rich Fibrin (PRF). Because of the solid fibrin gel polymerization of the preparation [20,21]. This method was so clearly not the same as different PRPs, that it was named a "second-age" platelet concentrate. [4] Although still not many ongoing examinations utilize a similar classification for both platelet thinks or still the writing have disarray to separate it. In 2012, the characterization framework for PRP and PRF was to a great extent referred to, supported and approved by a multidisciplinary agreement meeting and the arrangement was distributed in the exact year to annihilate the confusion [17]. Later in 2013, POSEID (Peri odontology, Oral Surgery, Esthetic and Implant Dentistry

Organization) hold it as rule for distribution on PRP and PRF [22].

#### 4. DIFFERENCE IN PRF, PRGF AND PRP

PRP is the original platelet concentrate. Nonetheless, the brief span of cytokine delivery and its poor mechanical properties have brought about hunt of new material. Tables 3 and 4

PRF is a characteristic fibrin - based biomaterial arranged from an anticoagulant - free blood reap with no fake biochemical modification (no cow-like thrombin is required) that permits fibrin layer enhanced with platelet and development factors. An in vitro examination demonstrated that PRF is better than PRP, thinking about the statement of basic phosphatase and enlistment of mineralization, caused especially by arrival of TGF- $\beta$  (Transformation Growth Factor) and PDGF-AB (Platelet Derived Growth Factor) [2]. The PRF protocol achieves the gel without any manipulation of the blood: this method, therefore, totally respects the European directive 2004/23/EC, while both the PRP and the PRGF require the addition of biochemical additives in order to be obtained.



**Fig. 3. History of PRF and PRP**

PRF is more preferred because of slow polymerization during centrifugation, fibrin based structure, ease of preparation, minimal expense makes PRF somewhat superior in some aspects of PFP. [2] Difference between the two material and advantages and disadvantages are elaborated in Tables 1,2,3 and 4 [23,24,25].

PRGF contains platelets in the pool of plasma. The ideal platelet include in PRGF is an inadequately characterized subject in the writing. Whitman et al. expressed that the platelet include in PRGF ought to sway somewhere in the range of 500,000 and 1,000,000 cells/L. Plasma wealthy in development factors (PRGF) is an

**Table 1. Based on processing**

<b>PRP (First Generation)</b>	<b>PRP (Second Generation)</b>
1. Use of Bovine Thrombin and Calcium Chloride (anticoagulant)	1. No anticoagulant required
1. Two spin centrifugation. Centrifuged – 1 <sup>ST</sup> spin – 1300 rpm for 10 minutes (soft spin)	2. Single Spin required. [4] (3000 rpm for 10 minutes)
2 <sup>nd</sup> spin – 2000 rpm for 10 minutes (hard spin) <sup>4</sup> (Fig. 2)	3. It involves speedy blood collection and immediate centrifugation.
2. After blood collection , one can wait for 10 minutes for centrifugation.	4. Preparation is simple.
3. Preparation is labour effective.	

**Table 2. On polymerization basis**

<b>PRP (FIRST GENERATION)</b>	<b>PRF (SECOND GENERATION)</b>
1. Sudden fibrin polymerization depending on the amount of surgical additives (Thrombin and Calcium chloride)	1. Slow natural polymerization on contact with glass particles of the test tube results in physiologic thrombin concentration.

**Table 3. Based on structure**

<b>PRP (FIRST GENERATION CONCENTRATE)</b>	<b>PRF (SECOND GENERATION CONCENTRATE)</b>
1. BILATERAL JUNCTIONS (Condensed tetra molecular) are constituted with strong thrombin concentration and allow the thickening of fibrin polymers leading to the constitution of a rigid network, unfavourable to cytokine enmeshment and cellular migration [24].	1. EQUILATERAL JUNCTION (Connected Trimolecular) allow the establishment of a fine flexible fibrin network able to support cytokines enmeshment and cellular migration. 2. This three dimensional organization gives great elasticity to fibrin matrix which is observed in flexible, elastic and very strong PRF membrane [24].

**Table 4. Based on biological property**

<b>PRP (FIRST GENERATION)</b>	<b>PRF (SECOND GENERATION)</b>
PRP has immediate release of growth factors.	PRF growth factors are released slowly over a period of 7 or more days [25].

autologous platelet concentrate which conveys a variety of polypeptide development factors advancing delicate tissue and bone regeneration [47]. The recuperating of periodontal pocket relates to pick up in connection of gingival epithelium on the root surface by cell multiplication and separation. Development factors delivered from PRGF advance these biologic cycles like multiplication, relocation and separation at the cell level. PRGF is a second era framework, like that of platelet rich plasma (PRP), utilized for getting platelets and plasma proteins and has extraordinary favorable circumstances as it needs less venous blood, is more advantageous, less tedious, protected, simple to utilize and initiates quicker mending.

## 5. CLASSIFICATION

Depending upon pharmacological and material characteristics of obtained product it is divided into 4 main families that is Pure -Platelet Rich Plasma (P PRP), Leucocyte- and Platelet Rich Plasma (L- PRP), Pure- Platelet Rich Fibrin (P-PRF) and Leucocyte-and Platelet Rich Fibrin (L-PRF). This classification should help to elucidate successes and failures that have occurred so far, as well as providing an objective approach for further development of these techniques [9]. This classification system was largely cited, advocated, and validated by a multi- disciplinary

consensus conference published in 2012. [17] The POSEIDO (Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization) hold it as its guidelines for all publications on the topic in 2013 [22]. According to classification it is divided as under:

### 5.1 Pure - Platelet Rich Plasma (P-PRP)

Products are preparation without Leukocytes and with low density fibrin network after activation. As per definition products are used as liquid solutions or gel. [4] There are two preparation methods automated and manual [9].

### 5.2 Leucocyte - and Platelet Rich Plasma (L- PRP)

Products are preparations with leukocytes and with a low density fibrin network after activation. The initial objective of developing alternative easy to handle method was to make it possible to use in daily practice without having the support of transfusion lab [9]. There are two preparation methods automated and manual.

### 5.3 Pure - Platelet Rich Fibrin (P-PRF)

It is also known as Leucocyte Poor Platelet Rich Fibrin. Products are without leucocyte and high density fibrin network. These products only exist

in activated gel form and cannot be injected or used like traditional fibrin glues. There is only one product in family commonly known as Fibrin PRFM by Cascade Medical. Main Disadvantage is cost as compared to L-PRF [13].

#### **5.4 Leukocyte-and Platelet Rich Fibrin (L-PRF)**

It is also known as Choukoun's PRF. Products are with leukocytes and with a high density fibrin network [14]. Choukroun's PRF protocol is a simple and free technique developed in France by Choukroun et al. [11] It can be considered as a second-generation platelet concentrate because the natural concentrate is produced without an anticoagulants or gelifying agents [12]. Unlike the PRPs, Choukroun's PRF does not dissolve quickly after application; instead, the strong fibrin matrix is slowly remodelled in a similar way to a natural blood clot. Platelets and leucocytes are collected with high efficiency in this method and leucocytes are preserved throughout [26,27]. This is considered as the best product because of low cost and easy procedure. Also considered best for daily practice. Table 5 regeneration.

### **6. PREPARATION**

There are four main parameter to be assessed in each platelet concentrate protocol. These are

1. Preparation kits and Centrifuge
2. Platelets and leucocytes
3. Fibrin [28] Table no. 5

All the platelet concentrates are also classified according to the method of processing. There are two methods mainly that is Automated and Manual. According to many scientific studies Manual is considered better [9].

#### **6.1 Leucocyte-poor or Pure Platelet-rich Plasma (P-PRP)**

Pure platelet concentrates for topical use were first developed as an additional application of the classical transfusion platelet units and were first reported for maxillofacial surgery [19].

*Automated protocols for P-PRP: plasmapheresis with a laboratory cell separator and Vivostat PRF*

The first technique of producing platelet concentrates for topical use was the so-called plasmapheresis, which uses a cell separator,

either in a discontinuous flow set up (in which the patients stays connected to the machine and the blood filtering continues until the desired quantity of platelets has been collected or starting from a bag of harvested blood with anticoagulant [29]. The different blood components, such as platelets, leucocytes and RBCs, are first separated from the PPP, which can then be reinfused to the patient. When the integrated optical reader detects the first buffy elements in the serum, these are automatically collected into a separate bag as the platelet concentrate (PRP). As soon as the optical reader detects elements of RBCs, platelet collection is interrupted and RBCs, mixed with leucocytes and some residual platelets, are directed towards a third separate collection bag before eventual reinfusion. This method allows around 40mL of PRP to be obtained from 450 mL of whole blood. With discontinuous flow, in which the patient stays connected to the machine, up to 300 mL of PRP could be collected.

#### **6.1.1 Manual protocols for modified P-PRP**

One of the first platelet concentrate protocols (PRGF, which stands for either plasma rich in growth factors [30] or preparation rich in growth factors [31] was Described in 1999 by Anitua and has been commercialized by BTI (BioTechnology Institute, Vitoria, Spain). In this protocol, venous blood is collected and centrifuged in several small tubes to obtain the three typical layers: RBCs, buffy coat and acellular plasma. The upper part of the acellular plasma is called plasma poor in growth factors (PPGF) and is discarded from each tube by careful pipetting to avoid creating turbulences. There main in plasma is termed PRGF and is collected with a pipette, using only eyeballing as a measuring tool. Several pipetting steps, each associated with possible pipetting and handling errors, are necessary to collect the entire PRGF fraction of the patient, after which fibrin polymerization is induced by a 10% calcium chloride solution. After 15 to 20 min, an unstable PRGF gel is formed that will need to be used immediately.

#### **6.2 Leucocyte-and Platelet-rich Plasma (L-PRP)**

##### **6.2.1 Manual protocols for L-PRP: Curasan, Friadent-Schutze, Regen and Plateltex**

Two similar protocols, each using a two centrifugation procedure, were marketed by Curasan and Friadent-Schutze respectively. A

**Table 5. Characteristic and classification**

FAMILY	PROCESS- ING	CENTRIFUGA- TION	HOLDING DURATION	COST	PLATELET COLLECTION	POLYMERIZA- TION
P-PRP	Automated Manual	Twice	Long	Automated is expensive. Manual is Inexpensive.	Automated is excellent. Manual is low.	Weak
L-PRP	Automated Manual	Twice	Long	Expensive	Good	Weak
P-PRF	Only Manual processing	Once	Long	Expensive	Good	Strong
L-PRF	Only Manual processing	Once	Long	Very Inexpensive	Excellent	Strong

first centrifugation separates' the blood component into three layers Buffy coat, RBCs and PPP. The Buffy coat and PPP are segregating leaving RBCs very carefully. And two are again centrifuged for second time that is second centrifugation. After the centrifugation, the PPP layer is discarded using the 'eyeballing' method. The PRP obtained is of high quantity composed of platelets, leukocytes and circulating fibrinogen, but it also contains few RBCs. The concentrate is applied with bovine thrombin and calcium chloride. The platelet protocol uses specific gelifying agents, such as Calcium gluconate and lyophilized purified batroxobin, an enzyme that cleaves fibrinopeptide, to induce fibrin polymerization without bovine thrombin and gelling in around 10 minutes. All these protocols require substantial manual procedures, meaning that procedures is time consuming, and furthermore, they only lead to small amount of L-PRP.[4] Details of Processing is given on Table no. 5

## 7. APPLICATION IN DENTISTRY

### 7.1 PRP and Wound Healing

In intense injuries, TGF-b1, PDGF, VEGF, bFGF, and EGF are seen at enhanced levels for bringing wound healing [32]. All of these elements are unconfined by platelets and leukocytes in PRP, so its application would enlarge their levels to quicken mending. Interestingly, in persistent injuries, the degrees of these equivalent development factors are decreased. Subsequently, PRP use would enhance these problematic levels to animate and quicken the mending cycle. To be sure, upgraded mending has been shown with PRP in ongoing and diabetic wounds [33].

### 7.2 PRP and Bone Repairing

Bone is a repeatedly remodelled structure with the osteoblasts creating and secreting the matrix and the osteoclasts breaking down the matrix in order to allow new bone formation. Osteoclasts are large, multinucleated cells that are of hematopoietic origin, differentiating from the monocyte-macrophage lineage [34], which are accountable for bone resorption at sites of microdamage. The maturation and distinction of osteoclasts is directly linked with that of osteoblasts, as a balance between bone resorption and matrix production must be maintained. Osteoblasts develop from mesenchymal stem cells (MSC) and are responsible for secreting the calcium phosphate matrix in bones [35]. One of the predicted roles of PRP is to stimulate the differentiation of MSC to osteoblasts to engender more cells that form new bone. Osteogenic growth factors in PRP, including TGF-b1 and BMP2 [36], help regulate the commitment of the progenitor cells to the osteoblast lineage.

### 7.3 PRF in Dentistry

#### 7.3.1 PRF and sinus lift

PRF can be used in two ways, either as fragments varied with different bone substitutes or as a sole filling material [37]. In Mazor and Simonpieri [37] studies, one or two PRF membranes were placed on the sinus membrane and osteotomy window as a sole filling material and no grafting material was used to fill the created space. Implants were placed spontaneously with sinus lift and serve as tent pegs. Tent pegs technique based on guided bone regeneration as implants are placed immediately with sinus lift [37,38]. Tajima placed also PRF clots, no membranes, as a sole filling



material. No complications were observed during the healing period.

### 7.3.2 PRF and regeneration of peri-implant bone defects

Platelet concentrates may not be relevant to improve osseointegration in normal conditions, but they may help for the regeneration of peri-implant bone defects. Three specific situations can be encountered. The first situation concerns the peri-implantitis also called deosseointegration. The second are provoked during implant placement, when the initial bone volume for implantation is not large enough for the support of implants. The last kinds of peri-implant bone defects can be encountered during an immediate post-avulsion or post-extraction implantation procedure. There have been few reports about a graft using PRF alone for peri-implant bone defects. Lee et al. demonstrated, in animal model, that peri implant defect sized 3.0 × 5.0 mm (width × length) was successfully repaired by the application of PRF alone in the bony defect. Only limited *in vitro* studies have been carried out on the effects of PRF on regeneration of peri-implant bone defects. There is a need for further studies to determine the behavior of PRF applied for use in critical sized bone defects in humans [39].

### 7.3.3 PRF and gingival recession

The root inclusion methods target covering the presented surface to enhance style, alleviate excessive touchiness just as challenges to keep up an ideal bucco-dental cleanliness. Coronally progressed fold method, with subepithelial connective tissue is the most prescient plastic strategy. As of late, so as to improve the proficiency of the root inclusion medicines and lessen the horribleness of the procedures (second careful giver site), different option are utilized, for example, the platelet rich fibrin (PRF) supplanting the connective tissue join. As per Aroca 39 PRF film expanded addition in width of keratinized gingiva at the test locales at a half year contrasted with the adjusted coronally progressed fold alone. In Jankovic study, the utilization of PRF layer in gingival downturn treatment gave satisfactory clinical outcomes at a half year contrasted with connective tissue unite (CTG) treated gingival downturns. No distinction could be found among PRF and CTG techniques in gingival downturn's treatment, aside from more prominent increase in keratinized tissue. Kumar exhibited that the autogenous platelet

concentrate unite (PCG) or subepithelial connective tissue join (SCTG), secured by a coronally situated fold, were successful in the treatment of shallow gingival downturn abandons (≥ 2 mm) with critical root inclusion (87% and 80% for SCTG and PCG, separately) at a year postoperatively. The clinical ramifications and points of interest of PRF film as unite material are identified with the shirking of a contributor site surgery and a significant lessening in quiet uneasiness during the early injury recuperating period. PRF can be considered as a suitable financially savvy choice. Further investigations are important to evaluate the histology of the recovered tissue [40].

## 7.4 PRGF in Dentistry

There are different investigations identified with PRGF in Dentsitry. Endodontics have less yet at the same time not many work has been finished. Investigation of Bakhtiar et al. exhibited the positive aftereffect of PRGF in complete zenith conclusion in two teeth and apical conclusion with proceeded with increment of dentinal divider thickness in two different cases at 22 months of subsequent period. [41]The utilization of PRGF has been shown as compelling in the treatment of intermittent aphthous stomatitis (RAS). The utilization of PRGF innovation in oral medical procedure has a place with an idea of "regenerative medical procedure". At the point when the outside of the dental embed is absorbed PRGF, fibrin layer is conformed to the embed, which delivers the development factors and improves a cycle of osseointegration [42]. Osteonecrosis The after effects of different examinations propose that treatment with PRGF can diminish the danger of creating Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) after tooth extraction in high-hazard patients going through the treatment with bisphosphonates [43].The infusion of PRGF following arthroscopy is more successful than the infusion of hyaluronic corrosive (HA) concerning the agony levels in patients with cutting edge inside confusion of the temporomandibular joint (TMJ) [44]. There are a few investigations upon the utilization of PRGF in periodontology. PRGF shows great outcomes in veiling the root surface, expanding the width of keratinized mucosa and covering of gingival recession [45,46].

## 8. CONCLUSION

This article aimed at simplifying the 3 concepts and uses in dentistry. And also increasing use of

PRP, PRF and PRGF in dentistry. The main goal of PRF, PRP and PRGF is low invasiveness and a high rate of clinical healing. Since the quality of platelet concentrates may vary according to the physical state of each individual, it is very difficult to compare the results of the so far published research. However, all three are useful and can be called as 'good healers'.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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