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Platelet Concentrates – A Panacea for Periodontal Regeneration

Sweta Pradhan[1-a], Neetha J Shetty[1-b*]

[1] *Department of Periodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Karnataka.*
**Corresponding author*

Review

History

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ABSTRACT

Periodontitis is an inflammatory condition of the periodontium leading to loss of supporting structures of the tooth. The regeneration of periodontium refers to the restoration of the lost tissues to their original form and function by reiterating the fundamental wound healing processes involved in their development. Research has led to the development of "Autologous biomaterials" provide signals for healing, repair, and regeneration. These autologous substances also fosters neo-angiogenesis and new bone formation and therefore have yielded encouraging results in the field of regenerative dentistry.

Platelet concentrates have higher concentration of growth factors which enhance periodontal regeneration. Blood derivatives have several advantages such as being autogenous, cost effective, less time consuming, simple to perform and prolonged release of growth factors. Since inception, many approaches have evolved also in-depth research has been done regarding its biological and clinical applications. Several modifications have been advocated in the conventional protocol like the advanced PRF, injectable PRF, PRF lysate and Titanium-prepared PRF.

This review paper addresses the evolution, applications of platelet concentrates for tissue engineering, recent advances, and novel protocols. Furthermore, several future perspectives of platelet concentrates, such as platelet concentrates as drug delivery agents, platelet dust and liposomes encapsulating platelet concentrates are also discussed.

Key words: Autologous biomaterials, periodontal regeneration, platelet concentrates, platelet rich fibrin.

a *swetapradhan53@gmail.com* *https://orcid.org/0000-0002-3604-9901* b *neetha.rajesh@manipal.edu* *https://orcid.org/0000-0001-8841-6235*

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Introduction

Periodontitis is an inflammatory condition of the periodontium leading to loss of supporting structures of the tooth. A periodontal regeneration refers to the restoration of lost tissues to their original form and function by reiterating the fundamental wound healing processes involved in their development.[1] Research in the field has led to the development of "Autologous biomaterials" that are naturally occurring substances in the body, which provide signals for healing, repair, and regeneration. Furthermore, synthetically generated "alloplastic materials" have also produced encouraging results in the field of regenerative dentistry. However, these alloplastic materials are avascular and may trigger a foreign body reaction.[2] Therefore, to enhance the healing and regeneration, autologous substances are preferred as they have an added advantage of fostering neo-angiogenesis and new bone formation.[3]

The two autologous biomaterials- platelets and fibrin, are believed to play integral roles in wound regeneration and healing.[4]

Platelets are small irregularly shaped cells derived from precursor megakaryocytes. They are approximately 2–3 mm in diameter, and consists of granules, mitochondria and prominent membrane structures. The alpha granules and lysosomes present in platelets are responsible for the expulsion of growth factors.[5] The Alpha granules also contain biologic mediators in abundance. Platelets get activated during tissue injury and release growth factors such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor and epidermal growth factor .[6] Due to their biological activity, platelets can create an accelerated chemo attractive gradient that recruits stem cells and facilitates regeneration. In recent years, blood proteins have led to the evolution of plasma concentrates enriched with growth factors like A-PRF, T-PRF, I-PRF, PRF lysates, and CGF and these autologous platelet concentrates offer hope for periodontal regeneration.

Evolution of Platelet Concentrates and Preparation Techniques[7]

The preparation of Fibrin Glue[7], Platelet Rich Plasma[7,8] and Platelet Rich Fibrin[9,10,11] has been depicted in Figure-01.

Fibrin Glues, Fibrin Sealants or Fibrin Tissue Adhesives

They are derived from human plasma and are analogous to the final stages of blood coagulation in which a fibrin clot develops.

The fibrin sealants are of two types:

- Homologous
- Autologous.

Plasma cryoprecipitate was used to prepare homologous fibrinogen concentrates, which was formulated by mixing two components, thrombin containing calcium ions and fibrinogen containing factor XIII. Fibrin sealants were first prepared from autogenous whole plasma, but later bovine thrombin was used to polymerize them to reduce the risk of infection transmission.[7]

Platelet Rich Plasma[7,8]

- CURASAN METHOD- Double centrifugation method (Figure-03)[9]
- The venous blood is drawn with an anticoagulant.
- The first centrifugation (''soft spin'') allows the blood to be separated into three distinct layers. Using a sterile syringe, the practitioner aspirates platelet rich plasma (PRP), platelet poor plasma (PPP), and some red blood cells. Then the material is transferred to another tube.
- The second tube will undergo a second centrifugation, which will be substantially longer and faster (a "hard spin"). Thus, platelets can be concentrated at the bottom of the tube and subsequently separated into three layers.
- Using a syringe, the practitioner can discard most of the PPP, leaving just enough serum to suspend the concentrated platelets. cPRP (concentrated platelet-rich plasma) is then prepared by gently shaking the unit.
- Bovine thrombin and calcium chloride is then added to cPRP.
- Platelet concentrate gels very rapidly, and fibrinogen is also collected during cPRP preparation, resulting in a fibrin matrix with especially interesting hemostatic and adhesive properties.

Platelet Rich Fibrin[10,11,12]

- Choukron's Protocol
- The research pioneer, Mr. Joseph Choukroun, has developed a 2nd generation platelet concentrate without the use of anticoagulants. After a single centrifugation cycle (3000 rpm, 10 minutes) it is possible to obtain (750 g) a platelet concentrate without coagulation factors from the superficial layer of centrifugation tubes[10]
- A fibrin matrix was formed after centrifugation of the formulation called Platelet rich fibrin. PRF is a concentrate of white blood cells, platelets, and fibrin.
- The initial PRF (also called **L-PRF**) was shown to contain 97% platelets and more than 50% leukocytes when compared with whole blood.
- The success of this technique depends solely on the speed at which the blood is collected and transferred to the centrifuge. To obtain a clinically useful clot from PRF, quick handling is absolutely necessary.

To address these limitations, several modifications are made and new forms of PRF were introduced.

The modifications are (Figure-02)[12]:

A-PRF

- The Low-Speed Centrifugation Concept was later developed to produce advanced PRF matrices in solid form.
- When compared to PRF, the A-PRF clot is more porous and contains more interfibrillar space.
- The sterile glass-based vacuum tubes were used for formation of A-PRF at slower speeds (1500 rpm) and for 14 minutes. [13]

A-PRF+

- In 2016, Kobayashi & co-workers suggested another modification of A-PRF by reducing the centrifugation time to 1300 rpm for 8 minutes.
- However, the PRF obtained in this manner is in the form of gel, which is not suitable for injection.
- To overcome this limitation, I-PRF was introduced. [14]

I-PRF

- Blood is drawn into tube without anticoagulants and centrifuged for three minutes at 700 RPM. In 2-4 minutes of centrifugation, the entire concentration of clotting factors and platelets reaches the top of the tube.
- Currently, it is mixed with bone grafts to form a gel-putty consistency with the particles incorporated into the bone graft. [15,16]

T-PRF

By centrifuging blood at 2800 rpm for 12 minutes in Titanium tubes, Titanium-PRF is obtained. As titanium has better hemocompatibility than glass and possibly led to a more polymerized fibrin, T-PRF offer a tighter, thicker fibrin than L-PRF.[17]

Other modifications include:

Concentrated Growth Factors Preparation [17]

- 9ml of venous blood was drawn and collected in sterile Vacuette tubes without anticoagulant solutions.
- Thereafter, the tubes were immediately centrifuged with the following characteristics: 30 sec acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 3 min at 3000 rpm, and 36 sec deceleration and stop.
- At the end of the process, three blood fractions were obtained:

(1) a superior phase represented by the serum (platelet-poor plasma, PPP)

(2) an interim phase represented by a very large and dense polymerized fibrin block containing the CGF, white blood cells and stem cells

(3) the lower red blood cell layer. The fibrin block with red interface was cut out as CGF

Autologous Fibrin Glue and Sticky Bone[18]

- To obtain Autologous protein glue (AFG), 20- 60CC of blood in non-coated tubes is centrifuged at 2400-2700 rpm for 2 minutes.
- Of the two layers obtained, the deeper layer contains RBCs, and the superficial layer contains AFGs.
- AFG is then extracted via syringe and mixed with bone powder and allowed to rest for 5-10 minutes for polymerisation, resulting in a very yellow coloured mass known as sticky bone.

Recent concepts-Albumin Gel-Platelet-Rich Fibrin Mixture (ALB-PRF) [19]

- 9 ml of blood was centrifuged at 700 g for 8 minutes.
- To produce albumin gel (denatured albumin), 2 ml of the uppermost layer (PPP layer) were collected and heated for 10 minutes at 75°C, followed by cooling for 10 minutes.
- Afterward, the liquid PRF, along with the residual cells and growth factors found within the buffy coat layer, was mixed with the albumin gel to produce **Alb-PRF**.
- Alb-PRF has higher growth factor content as compared to L-PRF. It contains PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, epidermal growth factor and insulin growth factor 1.
- In addition, it improves collagen synthesis, cell migration capacity, assays for proliferation.

BIO-PRF [20]

- **The horizontal centrifugation concept** was found by Miron *et al*. (2019).
- Miron *et al*. had stated that horizontal action resulted in formulation consisting of higher levels of platelets and leukocytes.
- The horizontal centrifuge has the advantage of separating layers based on density due to its design.
- There were two protocols used during this study, a solid-PRF protocol of 700g for eight minutes and a liquid-PRF protocol of 200g for 8 minutes.

Clinical Applications of Platelet Concentrates[7]

Platelet Concentrates and Periodontal Regeneration

Fibrin Sealants

- Fibrin sealants have been used in surgical procedures since the late 1970s.
- "Fibrin sealants" are plasma-derived human plasma substances that mimic the process of blood coagulation, forming fibrin clots, and that may be used for topical haemostasis, tissue sealing, and melting agents for particulate bone substitutes.
- Due to the risk of cross infection associated with commercial adhesives, autologous fibrin sealants made from the patient's own plasma were developed. Despite this, they exhibited inadequate rheological properties.[21]
- **Limitations [22]**
- Fibrin sealants have lower resistance to physical stress.
- Despite their documented benefits for soft tissues, it remain controversial when applied to periodontal surgery.
- Fibrin sealants may be associated with viral transmission risks.

Platelet-Rich Plasma (PRP)

- PRP mimics the clotting process at the end of the cascade of coagulation.
- Growth factors released through α-granules promote early wound healing via angiogenesis and cell proliferation.
- The three layers of PRP: - platelet rich plasma, platelet poor plasma, and red blood cells.[23]
- PRP has been investigated for its efficacy in various periodontal regenerative procedures through various animal experiments, clinical studies, randomised trials, systematic reviews and meta-analyses which is listed in Table01.[24-28]

Advantages of PRP:

1. The growth factors and cytokines present in PRP elicit rapid regeneration
2. There is no risk of disease transmission
3. it is convenient and cost effective for patients

Disadvantages of PRP:

1. No standardisation of PRP preparation protocol
2. Bovine thrombin added to platelet concentrates has been reported to cause adverse reactions such as systemic lupus erythematosus

PRP has become less popular due to the disadvantages listed above and has prompted the advent of PRF.

Platelet Rich Fibrin (PRF)

- It is formed during centrifugation because of natural polymerization.
- It has the unique property of slow-releasing growth factors for 28 days, including transforming growth factor-β, platelet-derived growth factor, vascular endothelial growth factor, and matrix glycoproteins such as thrombospondin.[30]
- It enhances regenerative and reparative processes. Moreover, it forms a trimolecular fibrin meshwork, facilitating cytokine function and cell migration.
- As PRF can lose structural integrity due to dehydration, it must be used immediately after preparation.
- PRF consists of three layers[31,32]

Advantages of PRF: 29

1. Ease of preparation and use
2. cost effective
3. Reduces the incidence of adverse reactions as there is absence of bovine thrombin or anticoagulant.

Disadvantages of PRF:

1. samples obtained from PRF have a relatively low quantity

- Numerous studies have revealed PRF's regenerative potential when applied to various periodontal procedures and they are described in Table no-02.[34-39]

Leucocyte Platelet Rich Fibrin

A modified PRF clot or membrane encapsulates most of the platelets and leukocytes derived from the initial blood harvest in addition to platelet growth factors and stem cells. (Figure-3)[40]

The use of L-PRF membrane for increasing the width of keratinized mucosa around implants has shown promising results in a randomized controlled trial by Temmerman *et al.*[41]

Advanced Platelet Rich Fibrin

- It is a modified form of pure PRF (Figure-4)[42]. The clot that results has an abundance of neutrophils and macrophages.
- These cells provide a matrix for cell migration into the defect and also contains biological factors that accelerate wound healing, such as platelet derived growth factor, transforming growth factor β, platelet factor 4, IL1, vascular endothelial growth factor, epidermal growth factor, endothelial cell growth factor, platelet-derived endothelial growth factor, insulin-like growth factor, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin. [42]
- Since it is a matrix obtained from the patient's own blood, foreign body reactions are eliminated. [42]
- Both L-PRF and A-PRF induced periodontal ligament fibroblast proliferation in an in-vitro study. The use of A-PRF for gingival recession treatment has shown significant results.[43]

Injectable Platelet-Rich Fibrin

- I-PRF is a liquid platelet concentrate that can be used alone or in combination with biomaterials. (Figure-5)[44]
- As compared to other forms of PRF, it was found to contain more regenerative cells and growth factors.
- Furthermore, it clots and develops a gel after approximately 10-15 minutes, which provides sustained growth factor release in the tissue, and has the potential to induce transforming growth factor-β and collagen-1 mRNA expression.
- In a study by Ozsagir *et al*., it has been observed that the application of I-PRF leads to increase gingival thickness in individuals with thin periodontal phenotypes.[44]

Titanium Platelet Rich Fibrin

- T-PRF was introduced by Tunali *et al*.[45] (2014), who hypothesized that titanium might have a greater effect on activating platelets than silica, which was previously used to prepare other PRF concentrates. (Figure 6)
- The regenerative potential of T-PRF has recently been evaluated in several randomized controlled clinical trials.
- The effects of T-PRF on periodontal parameters and radiographic changes have been reported in a study by Chatterjee *et al*.[45]
- When combined with open flap debridement, T-PRF membrane improved periodontal healing. 4 to 6 weeks after administration of T-PRF, it was concluded that the concentrations of growth factors were significantly higher and the RANKL/OPG ratio was significantly lower. [46]

Concentrated Growth Factors

- The CGF preparation process yields a much denser and larger fibrin matrix rich in growth factors. (Figure-7)[47]
- This matrix acts as an effective haemostatic agent.
- It accelerates osteogenesis and promotes wound healing.
- It improves wound stability by enhancing new connective tissue attachment.
- Moreover, it acts as a scaffold for cytokine attachment and cellular migration
- Researchers found an increase in gingival keratinized width and gingival thickness in response to CGF use in conjunction with coronally advanced flaps.[47]
- CGF is also used to treat intra-bony defects. In conjunction with GTR, it acts as an efficient barrier for bone regeneration.[48]

Autologous Fibrin Glue (AFG) And Sticky Bone

- It was introduced by Sohn (2010).
- Autologous fibrin glue was applied to bone graft which resulted in sticky bone, which was found to reduce post-operative complications and boost wound healing. (Figure-8)[49]
- It has been determined that "sticky bone" preparation can be used for alveolar ridge augmentation, since bone graft gets trapped within cross-linked fibrin meshwork which minimizes undesirable movement during healing. This increased tissue healing by stabilizing the bone graft without the use of bone tacks or titanium mesh. [49,50]

Future Perspectives

Platelets Concentrates as Drug Delivery Agents-

Platelets concentrates may serve as vehicles for loading drugs or biological therapies into specific targets. The ability of platelets to identify and interact with tumour cells has been demonstrated in studies. Therefore, developing new drug delivery systems and therapeutic strategies could be of use in the treatment of tumours since this might help to lessen the side effects of chemotherapy such as cytotoxicity. [10]

Application of Platelet Dust

Platelet microparticles (also known as "dust" or "PMPs") are among the most abundant cells-derived microparticles available. They are formed by the activation of platelets. These microparticles have drawn attention as potential diagnostic markers of various diseases. Research on these microparticles suggests they

can modulate immune systems and contribute to various diseases. [52]

Liposomes Encapsulating Platelet Concentrates-

By using advanced delivery systems such as liposomes to encapsulate platelet concentrates, it is ensured that the growth factors will remain intact for a longer period. It will also help in selective targeted delivery. The encapsulation of PRP in biodegradable scaffolds such as calcium phosphates and polylactic-co-glycolic acid has demonstrated positive results for enhanced bone regeneration in vitro studies. [53]

Conclusions

Using platelet concentrates as a regenerative procedure in periodontal surgery is a novel approach. Consequently, their ability to harbour growth factors makes them capable of accelerating periodontal tissue regeneration and enhancing wound healing. Considering it's an autologous, natural, and economic products, there is no risk of a reaction or disease transmission. The efficacy of treatment with platelet concentrates has been demonstrated in various studies, although large-scale studies are still needed to verify its efficacy.

Ethical approval letter and consent to participate

Not Applicable

Competing Interest

The authors declare that they have no competing interests in this section.

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None

Conflicts of Interest Statement

None.

Table-01. Studies of PRP

TRIALS	STUDIES
ANIMAL EXPERIMENTS	▸ A study conducted by Suaid et al revealed that when PRP and subepithelial connective tissue graft were used for the treatment of gingival recessions, the treatment was very effective at promoting new cementum formation. ▸ A study by Marcelo Diniz Carvalho et al showed that, when combined with bovine glass, the use of PRP failed to significantly improve periodontal regeneration in dogs with three walled intrabony defects.
CLINICAL TRIALS	▸ The results of a study conducted by Anitua showed improved soft tissue repair and bone regeneration after the application of PRP inside extraction sockets, indicating these sites could be prepared for implant placement in the future. An experimental study by Lekovic et al compared the ▸ effectiveness of bovine porous bone mineral combined with PRP and guided tissue regeneration in humans with intrabony defects. According to the study, PRP exhibited strong regenerative potential and resulted in reduced pocket depth and improved clinical attachment level.
RANDOMIZED CONTROLLED CLINICAL TRIALS	➢ Using PRP and a cancellous allograft to augment ridges, the Eskan et al study found that PRP enhanced bone regeneration.
SYSTEMATIC REVIEW AND META-ANALYSIS	➢ A systematic review by Panda et al evaluated the effectiveness of autologous platelet concentrates as well as other regenerative treatments in treating intrabony defects. The use of PRP in combination with grafting materials and guided tissue regeneration has been found to be effective but found to be ineffective when used alone.

Table-02. Studies of PRF

TRIALS	STUDIES
ANIMAL EXPERIMENTS	➢ Duan et al concluded that PRF combined with rat periodontal ligament stem cells could be useful for periodontal tissue engineering. ➢ According to an animal study conducted by Wang et al, lyophilized PRF and mesenchymal stem cell sheet fragments from osteogenic bone marrow promote bone tissue regeneration.
CLINICAL TRIALS	➢ In a study by Deepa and Jain (2015), PRF was shown to enhance healing of gingival fenestrations, which suggests its use as a membrane in aesthetically demanding areas.
RANDOMIZED CONTROLLED CLINICAL TRIALS	➢ Clinical parameters improved when PRF was used to regenerate intrabony and furcation defects, according to Pradeep et al. ➢ As a result of PRF combined with freeze-dried bone and a decalcified allograft, Agarwal et al. found that clinical and radiographic parameters had improved significantly after 12 months.
SYSTEMATIC REVIEW AND META-ANALYSIS	➢ The results of Strauss et al's systematic review suggest that PRF can increase implant stability and improve wound healing by reducing bone resorption after implant placement. ➢ In a meta-analysis by Li et al, it was determined that PRF and 1% alendronate were synergistically effective in regenerating periodontal tissues and bone.

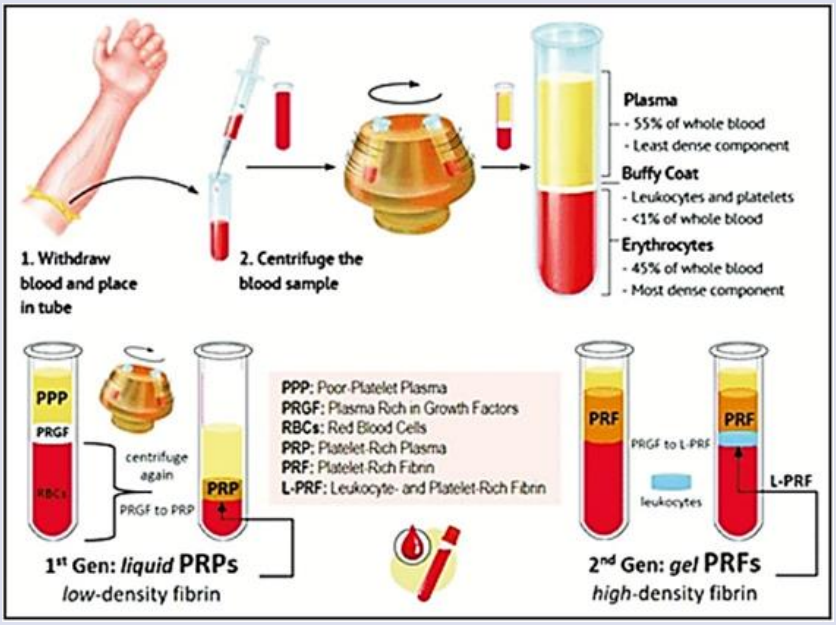

Figure 1. *PRP and PRF*

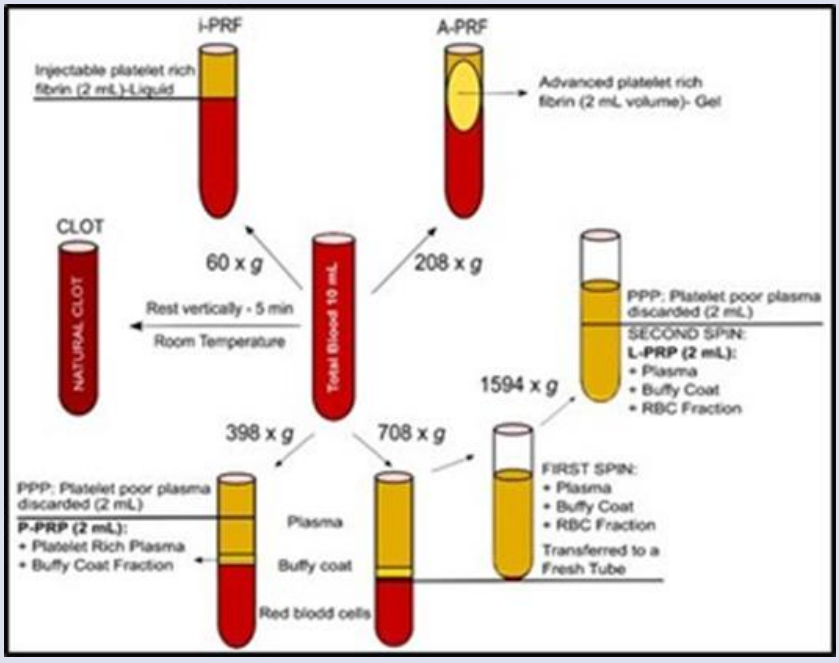

Figure 2. *Modifications of PRF*

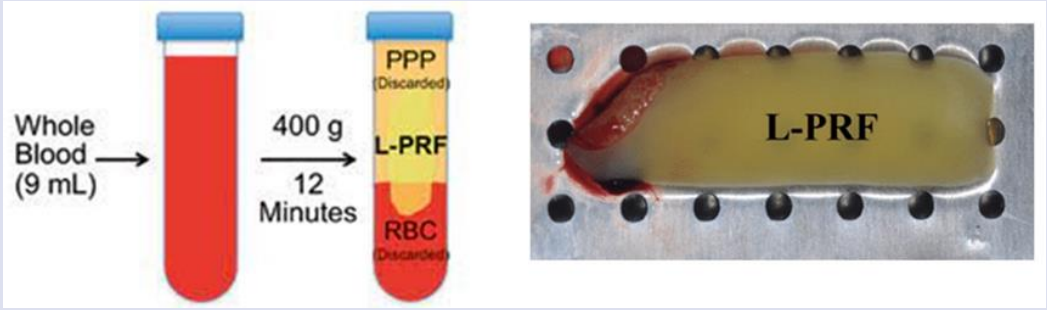

Figure 3. *L PRF*

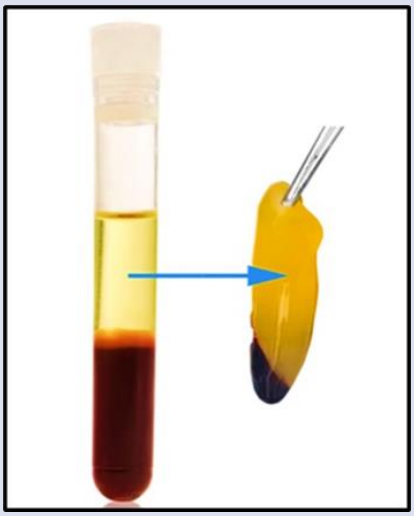

Figure 4. *A PRF*

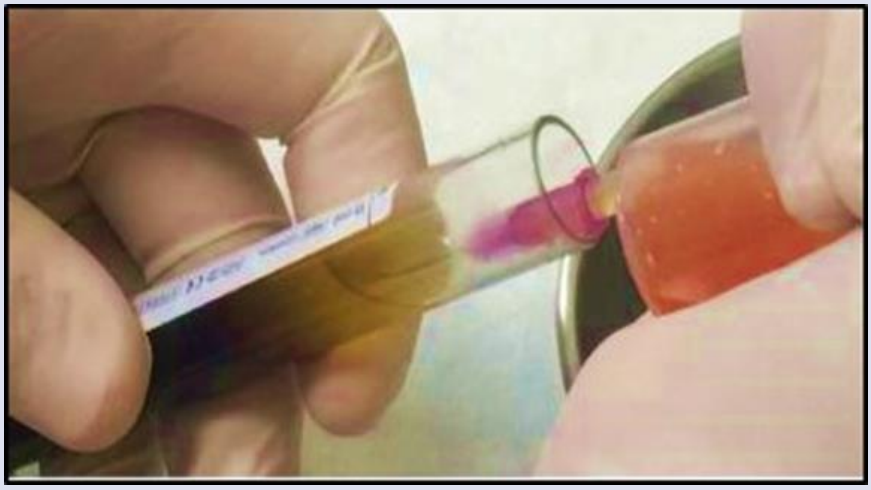

Figure 5. *I PRF*

Figure 6. *T PRF*

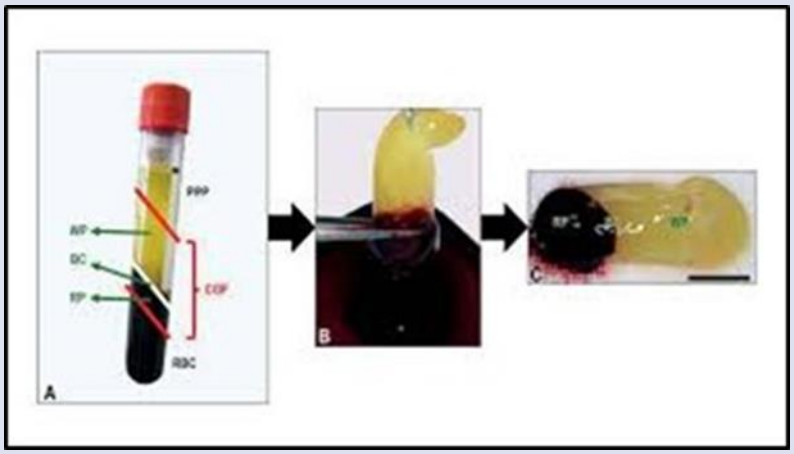

Figure 7. *Concentrated Growth Factor*

Figure 8. *Sticky Bone*

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