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Research Article

A BOON TO REGENERATIVE DENTISTRY: PLATELET CONCENTRATES

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A R T I C L E I N F O

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A B S T R A C T

The aim of any invasive procedure is the complete demolition and elimination of the infection and associated dead tissue followed by repair and regeneration of the affected tissue. So as to attain this goal varied biological merchandise are introduced, among that are blood platelet concentrates. Blood platelet concentrates with their higher concentration of platelets has been utilized in the sector of medication since the 1990s within the type of the first-generation concentrates-Platelet Rich Plasma, and also the second generation focused – Platelet Rich Fibrin. Choukroun's blood platelet rich fibrin is one such material that is employed by itself and additionally as an adjunct with grafts. It's been winning because it delivers high doses of growth factors and has medication properties. They need been shown to be of nice promise within the field of odontology, starting from implantology, sinus lift procedures, treating of odontology and dentistry lesions to regeneration of dead pulp. This novel technique has the potential to revolutionize the treatment mode in odontology and facilitate with reducing patient morbidity. We will be trying to look into the specification and implications of this material during this review.

INTRODUCTION

Periodontal disease could be an advanced, complex malady that is characterised by loss of connective tissue attachment and destruction of periodontal tissues. (Pihlstrom BL *et al*, 2005)

Aim of periodontal medical aid depends within the elimination of inflammatory method by preventing the progression of periodontitis and by regenerating the lost periodontium. (Chandrana P *et al*, 2014)

Regeneration is the replica or reconstitution of a lost or slashed half, in distinction to repair, that describes healing of a wound by tissue that doesn't absolutely reinstate the lost design or the function of that half. (American Academy of Periodontology, 2001)

Regeneration needs an orchestrated sequence of life events, like cell migration, adherence, growth, and differentiation, to possess potential to extend the success and predictability of regenerative procedures.

Tissue regeneration presently needs the idea of tissue engineering which incorporates three main components: cells, scaffolds (matrices), and signalling molecules (Fig. 1).

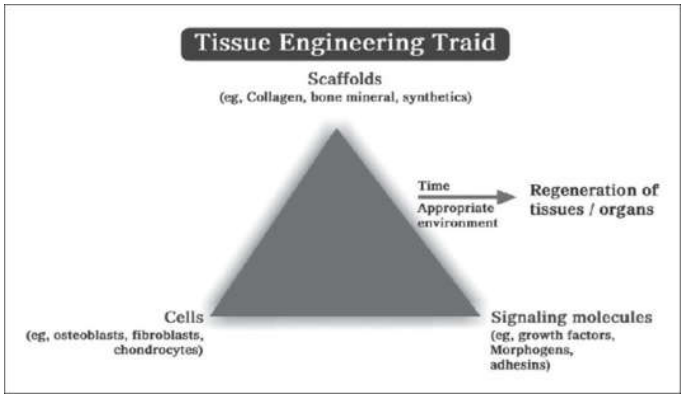

Fig 1 Tissue engineering triad

Signalling molecules contain growth factors that play a crucial role in regeneration. Growth factors are materials or proteins that have the potential to change key cellular events in tissue regeneration via binding to specific cell surface receptors.

Production and purification of growth factors is completed by deoxyribonucleic acid recombinant technology. As this can be an expensive and technique sensitive methodology, an alternative to provide growth factors is administered via natural process of blood centrifugation.

Blood could be a specialized humor. It's four main components: plasma, red blood cells, white blood cells, and platelets. Cells and platelets structure make up about 45% of human blood, whereas plasma makes up the opposite 55% of the entire blood volume (Fig. 2).

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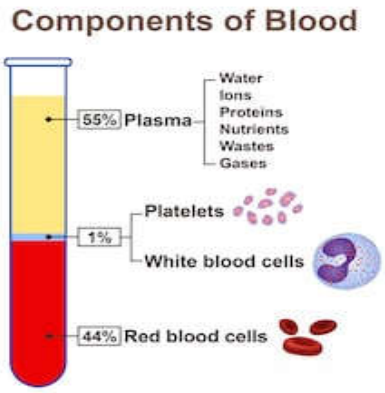

Fig 2 Components of blood

One of the most interesting breakthroughs in the field of regenerative dentistry is the revelation of platelet concentrates in which growth factors are entangled in fibrin network resulting in their release over a period of time, advancing the healing process of wound.

Platelets are anucleate cytoplasmic fragments obtained from bone marrow megakaryocyte which measures 2–3 µm in diameter.They are important in hemostasis and are a natural source of growth factors that are stored within platelet α-granules like the vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), platelet derived growth factor (PDGF), platelet derived angiogenic factor (PDAF), and transforming growth factor beta (TGF-β). The activation of platelets triggers the release of these growth factors. This is initiated by various substances or stimuli such as calcium chloride, collagen or thrombin. (Gassling VL *et al*, 2009)

Evolution of Platelet Concentrates

- Tayapongsak *et al*, 1994 added Autologous Fibrin Adhesive to cancellous bone during mandibular reconstructions that enhanced osteoconduction by virtue of the fibrin network.
- Whitman *et al*, 1997 used Platelet gel as an autologous alternative to fibrin glue for reconstructive oral and maxillofacial surgery.
- Robert Marx, 1998 pioneered in the field of PRP and bone grafting and introduced a technique for procurement of PRP, by sequestering platelets by gradient density centrifugation.
- Anitua *et al*, 1999 has commercialised pure PRP as Platelet Rich in Growth Factors (PRGF).
- De Obarrio *et al*, 2000 used Platelet gel in combination with DFDBA for the treatment of periodontal osseous defects.
- Choukran *et al*, 2001 developed second generation platelet concentrate- platelet rich fibrin (PRF).
- Okuda *et al* (2003) demonstrated that both PDGF-AB and TGF-β were highly concentrated in the PRP preparation.

Platelet Concentrates

Ross *et al* in 1974 established the regenerative potential and role of platelets in wound healing. (Ross R *et al*, 1974)

Platelet concentrates depends on a centrifugation process that works by putting the supernatants in rotation around a hard and fast axis, thereby applying associate acceleratory force perpendicular to axis. Relative force (RCF; G-force) is outlined because the quantity of acceleratory force that's applied to a sample during a centrifuge, directly proportional to the revolutions per minute (RPM) a sample during a test-tube is subjected to. (Hanna R *et al*, 2004)

This resultant force causes the separation of varied components within the sample supported the individual weight of its components and is that the basis for blood separation techniques allotted by laboratory centrifuges. RPM and RCF are connected by the formula RCF = one.12*r* (RPM/1000)² wherever, r is the centre of the centrifuge to tube end distance in millimeters. (Chandra RV *et al*, 2019)

Platelet Rich Plasma- First Generation Platelet Concentrate

Platelet rich plasma (PRP) was introduced by Marx *et al* in 1998 with exaggerated concentration of autologous platelets suspended in an exceedingly touch plasma after centrifugation. (Marx RE *et al*, 1998)
Processing Of P.R.P.

Autologous PRP was developed as a by-product of multiple element pheresis. Techniques and instrumentality have utterly improved since then.

3 main techniques obtainable for acquisition of PRP area unit as follow:-
Apheresis – Involves the removal of blood from a patient or donor in associate instrument, designed as a centrifuge during which elements of blood area unit quarantined. One amongst the elements is withdrawn and therefore the remaining elements area unit retransfused into the patient or donor. (Kassolis JD *et al*, 2000)

Procurement from one unit blood - this system uses one unit (350 ml) of the patient's blood, however rather than exploitation associate apheresis equipment, it uses a temperature-controlled centrifuge (cold centrifuge).

Blood that's obtained in an exceedingly transfusion bag is subjected to an occasional spin cycle of 1100 revolutions per minute for quarter-hour, which ends up in separation of the three fractions. once discarding the RBC fraction, the remaining two fractions area unit subjected to 4000 revolutions per minute for ten minutes to induce PRP. (Marx RE *et al*, 1998)

Procurement on a small scale - Recent studies have centered on using minimal amount of blood (10-50 ml) relying upon the procedure concerned, and a standard laboratory centrifuge for the acquisition of PRP.

This procedure uses double-spin centrifugation (2,400 revolutions per minute for ten minutes, so once discarding the RBC fraction, 3,600 revolutions per minute for fifteen minutes), and therefore the three elements area unit obtained in an exceedingly test-tube.

At the time of application of PRP, it is combined with associate equal volume of sterile saline solution that contains 10% calcium chloride (a citrate inhibitor required to coagulate plasma) and 100U/mL of sterile bovine thrombin (a substance that permits polymerization of the fibrin into an insoluble gel, that causes platelets to degranulate and release their mediators and cytokines); resulting in a sticky gel that is relatively easy to apply to the defects. (Okuda K *et al*, 2003)

Advantages of Autologous PRP

1. Autologous preparation.
2. Promotes stickiness and strength for clot stabilization.
3. Biologically acceptable to the basis surface.
4. Contains growth factors (PDGF & TGF-β) discharged by platelets.
5. Promotes ontogenesis.
6. it's haemostatic properties.
7. Contains a dense protein web that's extremely osteoconductive.
8. Contains high concentrations of leukocytes, that act as associate "autologous antibiotic", reducing the danger of infection.

Limitations of PRP

1. Lack of uniformity in PRP preparation protocol as totally different thrombocyte concentrations has different storage time.
2. Unharness of growth factors is for a shorter amount of your time.
3. Antibodies to bovine issue Va might cross react with human issue Va and might cause coagulopathies and rare injury episodes. (Marx RE *et al*, 2005)

Platelet Rich Fibrin- Second Generation Platelet Concentrate

One of the advantages of PRF over PRP is the inexpensive and easy protocol for the collection of the patient's blood along with the disuse of the Bovine thrombin and anticoagulants. (Raja VS, 2008)

Platelet rich fibrin is a second generation platelet concentrate, developed in France by Choukroun in 2001**,** is an autologous reservoir of growth factors which accumulates platelets and cytokines in a physiologic fibrin clot. (Choukroun J *et al*, 2001)

Processing of P.R.F

10ml of venous blood is collected in a glass tube followed by the centrifugation process, 3000RPM for 10 minutes. If not immediately centrifuged, diffuse polymerization of fibrin occurs, which results in a clot of reduced quantity and quality. (Kobayashi M *et al*, 2001)

After the processing of PRF, the sample of blood in the test tube is allowed to settle and separates into three layers:

The acellular plasma, also known as platelet-poor plasma (PPP) is the topmost straw-colored layer, lacking in platelet cells. This is followed by the PRF Clot that is rich in fibrin and has the growth factors and cytokines embedded in the polymerized structure.

The lower fraction is red and has RBC cells. The blood, when collected and placed in the test tube, undergoes the process of intrinsic coagulation on coming in contact with the glass, thereby separating the blood into the clot and the plasma. Hence, speedy blood collection and immediate centrifugation is advised for successful preparation of PRF. (Chowdhury S *et al*, 2013)

During centrifugation, the fibrinogen in the plasma fraction combines with thrombin and forms the PRF region that is found between the acellular plasma and the lower-packed RBC-rich fraction. The topmost acellular layer is discarded, the middle PRF layer is collected along with the attached RBCs from the test tube using pliers. The fibrin clot is then kept on a sterile surface, and the RBCs are tenderly scraped off. (Choukroun J *et al*, 2006) (Fig. 3)

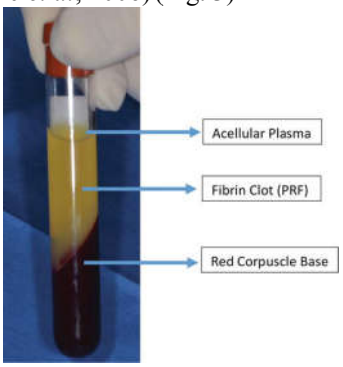

Fig 3 Platelet Rich Fibrin

The PRF Box is a device which is used for producing PRF membranes of uniform thickness in less than a minute (Fig. 4). The compression procedure is performed with a slow gentle and homogenous pressure, and the final membrane is always wet and soaked homogenously with the serum. There is no significant loss of extrinsic incorporated platelet growth factors and it has no influence on the intrinsic incorporated growth factor. (Ehrenfest DM *et al*, 2009)

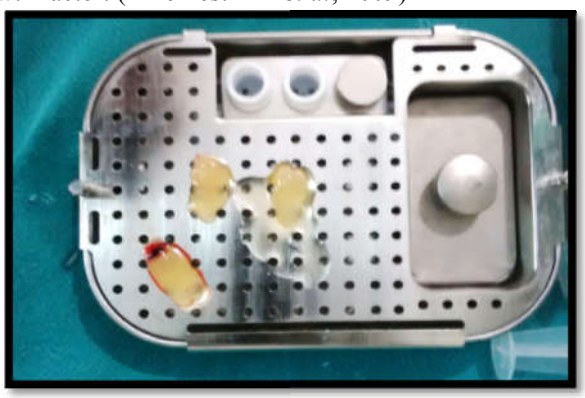

Fig 4 PRF Box

Advantages of using PRF *(Borie E et al, 2015)*

1. Simplified technique, with centrifugation in a single step.
2. Obtained by autologous blood sample.
3. Reduced blood manipulation.
4. No incorporation of external thrombin is needed as polymerization is a natural process, without any risk of suffering from an immunological reaction.
5. It has a natural fibrin framework embedded with growth factors that keep their activity for an extended period and stimulate tissue regeneration effectively.
6. It is used alone or together with bone grafts.
7. Is used as a membrane because it avoids donor site surgical treatment and causes token or no discomfort to the patient.

Disadvantages of using PRF *(Borie E et al, 2015)*

1. The final amount available is low because it is autologous blood.
2. The success of the PRF protocol depends directly on the handling, mainly, related to blood collection time and its transference for the centrifuge.
3. Need of using a glass-coated tube to achieve clot polymerization.

4. Short in vivo turnover rate.
5. Possible refusal of treatment by the puncture required for blood collection.

Recent Advances

Titanium - Platelet Rich Fibrin (T-PRF)- Third Generation Platelet Concentrate

T-PRF (Titanium prepared Platelet Rich Fibrin) is a third generation platelet concentrate developed by Tunali *et al* in 2014, prepared in grade IV titanium tubes in order to overcome the hazardous effect of silica present in the glass vacutainers.

T-PRF was obtained from centrifugation of 20 ml blood at 2800RPM for 12 minutes in grade IV titanium tubes. (Tunali M *et al*, 2013) (Fig. 5)

Fig 5 Titanium Prepared PRF

Advanced PRF (A-PRF)

Leukocyte and PRF (L-PRF) is produced at a speed of 2700 rpm for 12 minutes in sterile glass based plastic tubes. For formation of A-PRF, slower speed (1500 rpm) and more time (14 mins) are used in sterile plain glass-based vacuum tubes (A-PRF 10 tubes).

A-PRF protocol produced lighter, shorter, narrower clot with light polymerization and more squashed bodies. When the amount of growth factors (TGFβ, PDGF-AB, VEGF) released from A-PRF were compared to that of L-PRF, it was found that the levels were less than half of those from L-PRF. However, in another study, it was observed that A-PRF released significantly higher total quantities of growth factors when compared to traditional PRF. There is limited literature on the comparison between the two protocols and more studies are required to ascertain the benefits and limitations of L-PRF vs A-PRF. (Shah R *et al*, 2017)

Injectable PRF (i-PRF)

One of the recent developments in PRF technology is the production of injectable PRF (i-PRF). Injectable PRF (I-PRF) is the liquid form of PRF introduced by Choukran and Ghanati in 2014. (Fig. 6)

Fig 6 Injectable PRF

The rationale behind obtaining i-PRF is that it contains all components of PRF, including the platelets, white blood cells, and all the clotting factors comprising fibrinogen, in an uncoagulated form.

For producing i-PRF, blood is drawn without anticoagulant in plastic tubes without any coatings and centrifuged at around 700RPM for 3 minutes. Another set of authors have proposed a similar protocol where they centrifuge plain blood in non-coated test tubes at 2400-2700RPM for around 2 minutes. The supernantant is collected and they have named it concentrated growth factors (CGF). (Miron RJ *et al*, 2017)

Concentrated Growth Factors (CGF)

Unlike PRF using constant centrifugation (2700 rpm 12 mins) speed, CGF uses altered centrifugation speed (2400 – 2700 rpm 12 mins) that produce larger, denser and richer fibrin matrix containing growth factors. (Kumar P *et al*, 2015)

A standard, disposable, 10-ml non-anticoagulant tube and centrifuge device (MEDIFUGE, Silfradentsrl, S. Sofia, Italy) are used to obtain CGF (Fig. 7). Intravenous blood samples are collected from the patients and are placed in centrifuge tubes without anticoagulants 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. Due to the centrifugal device feature all of these acceleration and deceleration processes are adjusted automatically.

Fig 7 Medifuge Silfadentsrl

Three layers are observed in the tube: red blood cell 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 platelet-deprived fraction is removed with a sterile syringe. The layer containing the concentrated growth membrane is held with the help of a hemostatic clamp, separated from the red blood cell layer by cutting with a pair of scissors and then pressed to form a membrane. (Pirpir C *et al*, 2017)

Sticky Bone

The centrifugation protocol of Autologous Fibrin Glue (AFG) is 2400-2700RPM for 2 mins. Less centrifugation time leads to availability of more growth factors. (Kumar P *et al*, 2015)

Sohn *et al* 2010 fabricated growth factors enriched bone graft matrix and called it as sticky bone. Mixing of AFG to allograft or to mixture of allograft and xenograft produces yellow sticky bone. Addition of exudates from CGF to the above mixture produces red colored sticky bone. (Sohn DS *et al*, 2015) (Fig. 8)

Fig 8 Sticky bone

Albumin PRF (Alb-PRF)

Platelet-rich protein is an autologous membrane having benefits of host accumulation of platelets and leukocytes with growth factors within them. However, there are certain limitations of PRF that include quicker resorption properties (~2 weeks). It was seen in recent studies that by heating liquid platelet-poor plasma (PPP) layer, the resorption properties of heated albumin (albumin gel) get extended from a pair of weeks to more than four months To prepare Alb-PRF whole blood collected from peripheral blood in 9-mL plastic tubes is centrifuged at 700G for 8 minutes. Thereafter, the platelet-poor plasma layer is heated at 75° C for 10 minutes to create denatured albumin (albumin gel). The remaining cells and growth factor present within the buffy coat layer (liquid PRF) are mixed back with the cooled albumin gel to form Alb-PRF. It demonstrates a seamless release of growth factors up to 10 days with highest release of TGFβ1 followed by PDGF-AA and PDGF-AB. (Kobayashi MF *et al*, 2020) (Fig. 9)

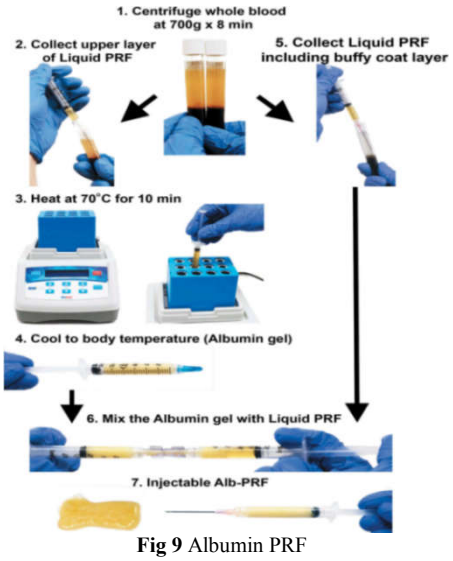

Fig 9 Albumin PRF

Applications of Platelet Concentrate

Platelet concentrates has immense use in the field of periodontology pertaining to its improved healing capacity, and better compatibility with the patient's immune system as it is purely autologous.

Periodontal surgeries are of mainly three types-

- Regenerative Therapy
- Periodontal Plastic Surgery
- Implants

Regenerative Therapy

In periodontal lesions such as infra bony defects and in regenerative procedures, along with endo-perio lesions, PRF can be used as a biological scaffold material. When compared to GTR membranes, PRF has been seen to promote periodontal progenitor cell proliferation and migration in situ. They also have inductive effect on gingival tissues due to the slow release of growth factors and cytokines. Since PRF membranes allow cell migration, they act as a barrier which stimulates the regenerative properties of periosteum by allowing neo angiogenesis and interactions between the gingival flap and alveolar ridges. (Kumar VR *et al*, 2015)

According to a study by Li *et al*, it was suggested that the fibrin component of the PRF played a substantial role in the osteo-differentiation as compared to the periodontal progenitors. The study also demonstrated that the PRF augmented the periodontal regeneration in two ways:

- Initiate soft tissue healing causing modification by progenitor proliferation and migration.
- Induction of alveolar bone facilitated by the fibrin mediated effect on RUNX2 expression, osteoblast differentiation and matrix mineralization along with the increase in alkaline phosphatase activity. (Li Q *et al*, 2013)

In the treatment of surgical endodontics such as periapical lesions and regeneration of pulp (in a case of tooth with a previously necrotic pulp with an open apex), PRF is used as an ideal scaffold material for repair and regeneration of the tissues since it acts as a matrix for the migration of cells. PRF also promotes revascularization of teeth due to the release of growth factors. (Chowdhury S *et al*, 2013)

Freeze Dried Bone Allograft (FDBA) when used along with PRF is found to accelerate the bone regeneration in cases of sinus augmentation. The healing time has been found to reduce considerably. Also, the use of PRF increases the graft volume and reduces the amount of bone harvesting. The use of PRF does an important role in the success of the graft by supporting angiogenesis and promoting revascularization. (Choukroun J *et al*, 2006)

Periodontal Plastic Surgery

In post-surgical procedures to complement healing of donor sites, PRF membrane, which is obtained after compression of PRF, can be used to reduce the healing time of the donor site. The PRF membrane provides a solid, stable fibrin mesh which is more rigid than the blood clot. The presence of growth factors such as PDGFs and TGFs accelerates the proliferation and migration of fibroblasts inside the wound. (Albanese A *et al*, 2013)

Use of PRF membrane in gingival recession treatment provided acceptable clinical results, followed by enhanced wound healing and decreased subjective patient discomfort compared to CTG treated gingival recessions.

Implants

Tatullo *et al* studied the effects of PRF in reconstructive surgery of atrophied maxillary bones and found that there was a clinical success rate of 87.5 to 100% in sinus lift by using PRF obtained following Choukroun's protocol. It was also seen that the use of PRF along with piezo surgery reduced the healing time, thereby achieving good primary stability of endosseous implants. The use of PRF allows us to avoid the use of membranes and barriers, and hence, reduce the risk of possible exposure to the oral cavity and infection by the oral flora. (Tatullo M *et al*, 2010)

In the new and ever developing field of implantology, such as reconstructive implant surgery, socket preservation, treatment of fenestration defects around implants, when it comes to implant placement, uneventful extraction is essential along with bone regeneration around the implant site and improved healing of the gingiva for the success of an implant. In such cases, PRF owing to its simple, inexpensive and less time-consuming preparation is considered an ideal material to be used as plugs for filling of extraction sockets even under conditions of socket destructions because of cysts. It can also be used along with bone substitutes, and as a protective biological barrier membrane, thus promoting gingival healing in cases where gingival wound closure is difficult to attain even with sutures. (Choukroun J *et al*, 2006)

Mazor *et al* assessed the relevance of autologous leukocyte, and platelet-rich fibrin (PRF) concentrate and membranes as the sole filling material during a lateral sinus lift with immediate implantation in a case series. From a radiologic and histologic point of view at six months after surgery, the use of PRF brought about a high volume of naturally regenerated bone in the sub sinus cavity. Further, they concluded that Choukroun's PRF is a simple and inexpensive biomaterial, and its systematic use during a sinus lift seems a relevant option. (Mazor Z *et al*, 2009)

CONCLUSION

The platelet sequestration method ends up in a high platelet concentrate that upon activation releases a cascade of growth factors contained within the alpha granules.

Growth factors discharged from platelets appear to signal the native mesenchymal cells to migrate, divide, and increase collagen and matrix.

Platelet concentrates as an entire have shown to own nice scope within the field of reconstructive and regenerative dental medicine. PRP is associated with autologous preparation, so it eliminates concern about disease transmission. Being the foremost recent of blood platelet derivatives, is safer and less complicated than PRP concentrates thus, they're simply used clinically. The healing and regenerative properties of the PRF are attributed to its basic fibrin composition. This autologous fibrin matrix has the power to release cytokines over a amount of 7-11 days at the side of the slower release of growth factors, that helps in reducing the healing time.

The 3 dimensional design of the fibrin matrix additionally helps in higher wound healing by an efficient direct cell migration. Additionally the elastic nature of the fibrin matrix permits the practitioner to manipulate the material according to use. It additionally helps in reducing the shrinkage and necrosis of flap, maintenance of the flap in an exceedingly stable position and wound coverage as a result of the mechanical adhesive property of the fibrin matrix.

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