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REVIEW ARTICLE

ADVANCEMENTS IN REGENERATIVE ENDODONTICS: PLATELET-RICH PLASMA (PRP) AND PLATELET-RICH FIBRIN (PRF)

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ABSTRACT

Pulp vitality is very important for tooth viability, as it provides nutrition and act as alarm to detect pathogenic stimuli. Regenerative endodontic procedures aim at regeneration of pulp and dentin of the affected teeth. The aim of this review is to add a new prospect in regenerative endodontic therapy by using platelet-rich plasma (PRP) and platelet-rich fibrin (PRF). PRP and PRF have an intimate assembly of cytokines, glycan chains and structural glycoproteins which are enmeshed in polymerized fibrin network, and have the potential to accelerate soft and hard tissue healing. Thus this review is intended to add light on the various prospects of PRP and PRF and clinical insights to regenerative endodontics.

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INTRODUCTION

The advancement of science and technology has huge impact on the present day world. It has contributed greatly on every aspect of our lives, including medical and dental care. One of such achievement is regenerative therapy, as it provides hope of converting non-vital tooth into vital once again and modern exploration of regenerative dentistry has added impetus onto the field of molecular biology. The American Association of Endodontists' Glossary of Endodontic Terms (2012) defines regenerative endodontics as "biologically based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentin complex"(Bansal *et al*., 2015). In the last decade, different sources including dental follicles, apical papilla, exfoliated deciduous teeth pulp, permanent teeth (premolars, molars) pulp and PDL have been investigated for mesenchymal Stem Cells (MSCs) isolation (Akiyama *et al*., 2012; Hakki *et al*., 2014; Hakki *et al*., 2015; Huang *et al*., 2009; Gosau *et al*., 2013). Platelets can play a crucial role in periodontal regeneration as they are reservoirs of growth factors and cytokines which are the key factors for regeneration of bone and maturation of soft tissue (Schliephake, 2002; Sunitha Raja and Munirathnam Naidu, 2008; Cole *et al*., 2010). Platelet–rich fibrin (PRF) and Platelet-rich plasma (PRP) are autologous platelet concentrates that are prepared from patient's own blood and are widely used in regenerative endodontics. That is why, this review article is intended to add light on various prospects of PRF and PRP and make the dental fraternity aware of these regenerative endodontic procedures and their various applications in dentistry.

Overview of regenerative endodontics: Regenerative medicine includes tissue engineering, genetic engineering and molecular activators. The principles of regenerative medicine can be applied to endodontic tissue engineering. Regenerative endodontics comprises research in adult stem cells, growth factors, organ-tissue culture, and tissue engineering materials

Tissue engineering: Tissue engineering is an interdisciplinary field that integrates the principles of biology and engineering to develop biological substitutes that replace or regenerate human cells, tissue or organs in order to restore or establish normal function (Howard *et al*., 2008).

There are three key elements for tissue engineering

- Stem cells-to respond to growth factors.
- Scaffold of extracellular matrix (ECM).
- Growth factors (signals for morphogenesis)

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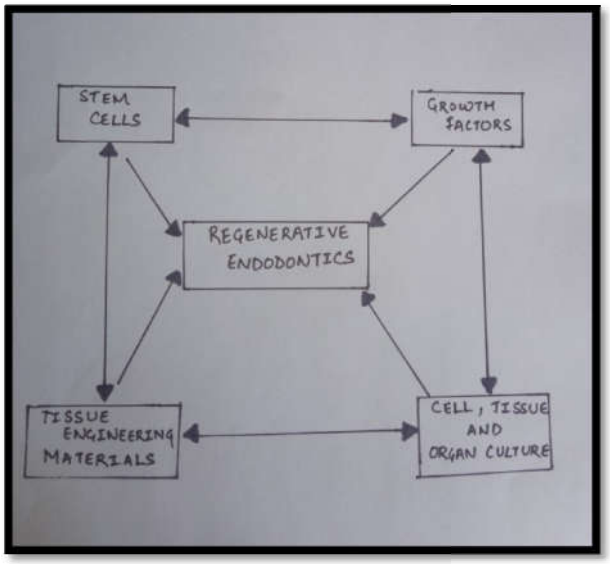


Fig.1. The major domains of research required to develop regenerative endodontic procedures

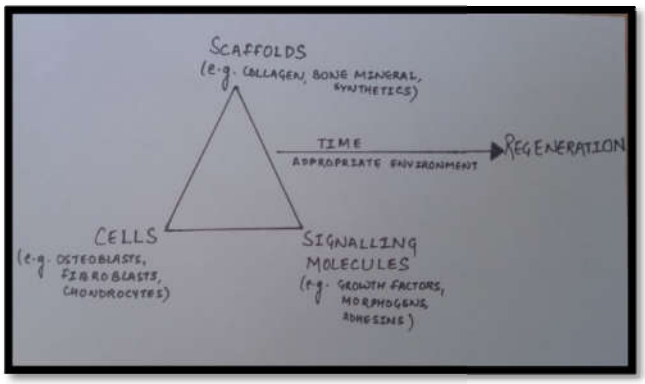


Fig. 2. Triad of tissue engineering

Stem cells: They are defined as clonogenic cells capable of both self-renewal and multiline age differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity.

Classification of Stem Cells

On the basis of origin

- Embryonic stem cells (ES Cells)
- Somatic/ Adult/ Post-natal/ Mesenchymal stem cells (AS Cells)

On the basis of Source

Autologous: Obtained from same individual to whom they will be implanted.

Allogenic: Obtained from the donor of same species.

Xenogenic: Obtained from the donor of another species.

Syngenic/ Isogenic: Obtained from genetically identical organisms, twins, clones or highly inbred research animals.

On the basis of Potency (Range of differentiation)

Totipotent: can differentiate into all embryonic and extra embryonic cell types.

Pluripotent: can differentiate into all types of cells except cells of the embryonic membrane.

Multipotent: can differentiate into more than one mature cell.

Unipotent: can differentiate into only one type of cells.

Scaffold: A Scaffold is thought of as a 3-dimensional construct or support substance used for several tissue engineering applications. The primary function of the scaffold is to provide a template to introduce the progenitor MSCs to the specific site of interest and to provide interim mechanical stability for tissue growth and integration.

Types of scaffold

Biological/natural scaffolds

Examples: collagen, silk, dentin, chitosan, fibrin and Platelet rich plasma.

Artificial scaffolds: These are synthetic polymers like polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers, polylactic-co-glycolic acid (PLGA), hydroxyapatite (HA), tricalcium phosphate (TCP), bioceramics, titanium and calcium polyphosphate (CPP).

Growth factors: Growth factors are extracellularly secreted signals governing morphogenesis /organogenesis during epithelial-mesenchymal interactions. They regulate the division or specialization of stem cells to the desirable cell type, and mediate key cellular events in tissue regeneration.

Methods of regenerative procedures

The various regenerative endodontic techniques are

- Root canal revascularization via blood clotting
- Postnatal stem cell therapy
- Pulp implantation
- Scaffold implantation
- Injectable scaffold delivery
- Three-dimensional cell printing
- Gene delivery.

Platelet-rich plasma (PRP)

Definition: Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline (Mehta and Watson, 2008; Marx, 2001). PRP also has been referred to as platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel and platelet releasate.

Classification: Four main generations of preparations can be defined, depending on their cell content and fibrin architecture.

Pure Platelet-Rich Plasma (P-PRP) or **leucocyte poor PRP:** Products are preparations without leucocytes and with a low-density fibrin network after activation.

Leucocyte and Platelet-Rich Plasma (L-PRP): Products are preparations with leucocytes and with a low density fibrin network after activation. It is in this generation that the largest number of commercial or experimental systems exist.

Pure platelet-rich fibrin (P-PRF) or **leucocyte poor platelet-rich fibrin:** Preparations are without leucocytes and with a high-density fibrin network.

These products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues.

Leucocyte and platelet-rich fibrin (L-PRF): Or second-generation PRP products are preparations with leucocytes and with a high-density fibrin network.

Components of PRP

The specific components of PRP are

- Platelet derived growth factor (PDGF)
- Transforming growth factors (TGFs)

Both of these are contained in the alpha-granules of platelets

- Fibronectin
- Vitronectin
- Fibrin

These are called cell adhesion molecules found in plasma. Platelet-derived growth factor (PDGF) is the evolutionary sentinel growth factor that triggers the wound healing process.

It exists in three isomeric forms

- PDGF-AA
- PDGF-BB
- PDGF-AB

Mechanism of action: PRP functions as a tissue sealant and drug delivery system (Eppley *et al*., 2004), with the platelets initiating wound repair by releasing locally acting growth factors (Everts *et al*., 2006; Knighton *et al*., 1986; Knighton *et al*., 1988). Via α- granules degranulation. Platelets in PRP also play a role in host defense mechanism at the wound site by producing signalling proteins that attract macrophages.

Preparation and activation of PRP: PRP is obtained from a sample of patients' blood drawn at the time of treatment. A 30 cc venous blood draw will yield 3-5 cc of PRP depending on the baseline platelet count of an individual, the device used, and the technique employed.

The blood draw occurs with the addition of an anticoagulant, such as citrate dextrose A to prevent platelet activation prior to its use. Activation is done by adding thrombin or calcium to the concentrate resulting in a gelatinous platelet gel. There are many ways of preparing PRP. It can be prepared by the PRP method or by the buffy-coat method (Sweeny and Grossman, 2002; Welsh, 2000).

Buffy coat method

- WB should be stored at 20°C to 24°C before centrifugation.
- Centrifuge WB at a 'high' speed.
- Three layers are formed because of its density: The bottom layer consisting of RBCs, the middle layer consisting of platelets and WBCs and the top PPP layer.
- Remove supernatant plasma from the top of the container.
- Transfer the buffy-coat layer to another sterile tube.
- Centrifuge at low speed to separate WBCs or use leucocyte filtration filter.

Clinical applications: (Froum *et al*., 2002; Arora *et al*., 2009)

- Endodontics: PRP has been used successfully in the treatment of periapical lesions, infected necrotic pulp tissues, open apex.
- Ridge augmentation can be achieved with the use of PRP.
- In sinus lift procedures, PRP accelerates the healing and reduces the healing time with stable bone gain.
- Socket preservation to maintain the alveolar bone height is possible.
- Intrabony defects or osseous defects have shown bone fill with the use of PRP.
- Jaw reconstruction surgeries.
- Soft tissue procedures like gingival grafts, sub epithelial grafts, and so forth, because of the property of PRP of accelerating soft tissue healing.

Commercially available PRP kits: PRP devices can be usually divided into lower (2.5-3 times baseline concentration) and higher (5-9 times baseline concentration) systems. The high-yielding devices include Biomet GPS II and III (platelet count 3-8×); Harvest SmartPRep 2 APC+(4-6×); ArterioCyte-Medtronic Magellan (3-7×). The lower concentration systems include Arthrex ACP (2-3×), Cascade PRP therapy (1-1.5×) and PRGF (2-3×) (21-25).

Platelet-rich fibrin

WHAT IS PRF? PRF is often named as Choukroun's PRF after its inventor (Dohan *et al*., 2006; Choukroun *et al*., 2001). Who invented this in France in 2001. It is a second-generation platelet concentrate.

PRF preparation: The procedure involves drawing of blood that is collected into test tubes without an anticoagulant and needs to be centrifuged instantaneously. A tabletop centrifuge can be used for this purpose at 3,000 rpm for 10 minutes. The resultant product consists of the three layers (Fig. 3).

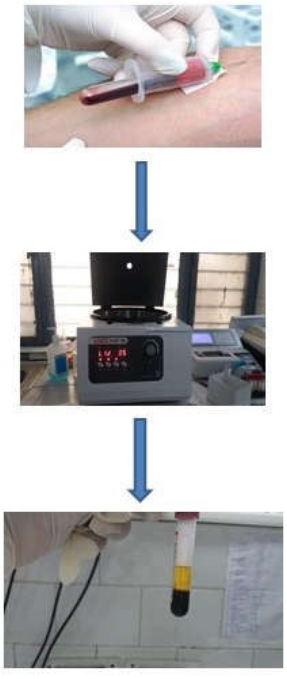


Fig. 3. Flowchart describing preparation of PRF

- Straw coloured fraction of acellular platelet poor plasma (PPP) at peak level
- PRF clot in intermediate level
- Red fraction of red blood cell (RBCs) at the base level

Recently, PRF box (Process, Nice, France) has been announced. It is formulated to produce homogenously thickened hydrated membrane and an exudate rich in platelets, leukocytes, vitronectin and fibronectin expressed from the fibrin clots. It has improved the issues regarding the handling of the PRF clot (28).

Oral implications of PRF

- PRF and PRF membrane have been used in combination with bone grafts to hasten the healing in lateral sinus floor elevation procedures.
- Protection and stabilization of graft materials during ridge augmentation procedures.
- Socket preservation after tooth extraction or avulsion.
- Regenerative procedures in treatment of 3-walled osseous defect.
- It should be in the treatment of combined periodontic-endodontic lesion
- Treatment of furcationdefect.
- PRF enhances palatal wound healing after free gingival graft.
- Filling of cystic cavity

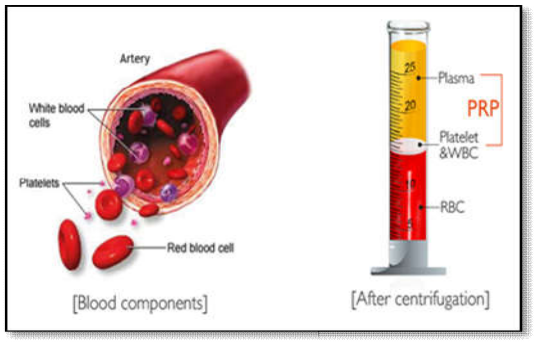
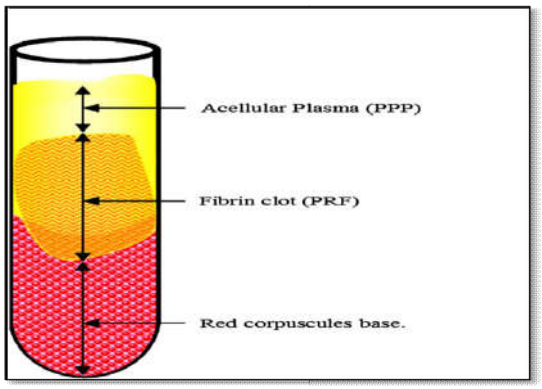
Regenerative endodontic applications of PRF

- Shivashankar *et al*. described a case report highlighting the combined use of graft material (PRF and hydroxyapatite [HA] and barrier membrane in the treatment of large periapical lesion (Shivashankar *et al*., 2013).
- Jayalakshmi *et al*. used PRF in combination with beta tricalcium phosphate (β-TCP) bone graft in the treatment of periapical cyst (Jayalakshmi *et al*., 2012).
- Keswani *et al*. reported that PRF might serve as a potentially ideal scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors, enhances cellular proliferation and differentiation, and acts as a matrix for tissue ingrowth (Keswani and Pandey, 2013).
- Huang *et al*. conducted an investigation into the biological effects of PRF on human dental pulp cells (Huang *et al*., 2010).
- Hiremath *et al*. reported affirmative results with pulpotomy using PRF (Hiremath *et al*., 2012).

Conclusion

However, many teeth are not given the opportunity to be saved by endodontic treatment and instead are extracted, with subsequent placement of an artificial prosthesis, such as an implant.

Table 1. Differences between prp & prf

S. No.	Platelet-rich plasma (PRP)	Platelet-rich fibrin (PRF)
1)	Introduced by Whitmen et al in 1997	Introduced by Choukroun et al in 2001
2)	Preparation of PRP is a two-step procedure **1st step**-PRP is produced by separation of platelet concentrate from platelet poor plasma, white & red cell fraction (2400 rpm for 10 minutes) **2nd step**-Exogenous thrombin or other activators like calcium chloride or calcium gluconate is added (3600 rpm for 10 minutes)  	Preparation of PRF is a **single step** procedure without adding any anticoagulant i.e. RBC base layer, acellular plasma on top & PRF clot in the middle (at 3000 rpm for 10 minutes) 
3)	The preparation of PRP takes more time	Chair side preparation of PRF is easy and fast
4)	Preparation of PRP is more technique sensitive	Preparation of PRF less technique sensitive
5)	There is drastic activation due to addition of calcium chloride etc. & rapid polymerization	Slow physiological polymerization
6)	The structure of PRP consists of dense network of monofibres	The structure of PRF is flexible three-dimensional fibrin network
7)	Concentration of cytokines in PRP is less	Increased concentration of cytokines in PRF
8)	PRP shows weaker healing kinetics	PRF has consistent & stronger healing kinetics
9)	PRP basically shows osteopromotive mechanism i.e. 81% of total TGF-β1 & similar levels of PDGF-AB within 1st day with significantly decreased release at 3,7 & 14 days.	PRF releases its growth factors steadily with the peak level reaching at 14 days corresponding to the growth pattern of periapical tissues
10)	PRP generates strong proliferation, but inhibits differentiation of bone mesenchymal cells (BMSC)	PRF shows proliferation & differentiation of BMSC
11)	PRP has poor mechanical properties because of its liquid or gel form	PRF has comparatively good mechanical properties
12)	PRP dissolve readily after application	PRF does not dissolve quickly after application, instead it is remodelled slowly in a similar way to a natural blood clot

Regenerative endodontic methods have the potential for regenerating both pulp and dentin tissues and therefore may offer an alternative method to save teeth that may have compromised structural integrity. The advances in regenerative endodontics comprise of Platelet-rich plasma (PRP) and Platelet-rich fibrin (PRF). PRP is a new application of tissue engineering and can be used in varied areas of the dentistry. It is a storage vehicle for growth factors, especially PDGF and TGF-β. However, high cost, limited volume of drawn blood, differences in centrifugation guidelines, wide variation in platelet concentrations led to the evolution of PRF. Properties of PRF like being completely natural, physiologic and comparatively economical source of autologous growth factors and cytokines makes it a desirable treatment option. Regenerative endodontics is an inevitable therapy and resources should be utilised for its development. The unleashed potential of regenerative endodontics may benefit millions of patients each year.

REFERENCES

Akiyama, K., Chen, C., Gronthos, S. and Shi, S. 2012. Lineage differentiation of mesenchymal stem cells from dental pulp, apical papilla, and periodontal ligament. *Methods MolBiol.,* 887:111–21.

Arora, N.S., Ramanayke, T., Ren, Y.F. and Romanos, G.E. 2009. Platelet-rich plasma: a literature review. *Implant Dentistry,* 18:303-8.

Bansal, R., Jain, A. and Mittal, S. 2015. Current overview on challenges in regenerative endodontics. *J Conserv Dent.,* 18:1-6.

Choukroun, J., Adda, F., Schoeffler, C. and Vervelle, A. 2001. An opportunity in perio-implantology: the PRF (in French). Implantodontie, 42:55-62.

Cole, BJ., Seroyer, ST., Filardo, G., Bajaj, S. and Fortier, LA. 2010. Platelet-rich plasma: Where are we now and where are we going? *Sports Health,* 2:203-10.

Dohan, DM., Choukroun, J., Diss, A., Dohan, SL., Dohan, AJ., Mouhyi, J. and Gogly, B. 2006. Platelet-rich fibrin (PRF): a second generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.,* 101:e37-44.

Eppley, BL., Woodell, JE. and Higgins, J. 2004. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg.*, 114(6):1502-8.

Everts, PA., Brown Mahoney C., Hoffmann, JJ., *et al*., 2006. Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors,* 24(3):165-71.

Froum, S. J., Wallace, S. S., Tarnow, D. P. and Cho, S. 2002. Effect of platelet-rich plasma on bone growth and Osseointegration in human maxillary sinus grafts: three bilateral case reports. *International Journal of Periodontics and Restorative Dentistry*, 22:45-53.

Gosau, M., Götz, W., Felthaus, O., Ettl, T., Jäger, A. and Morsczeck, C. 2013. Comparison of the differentiation potential of neural crest derived progenitor cells from apical papilla (dNC-PCs) and stem cells from exfoliated deciduous teeth (SHED) into mineralising cells. *Arc Oral Biol.,* 58:699–706.

Hakki, SS., Bozkurt, SB., Hakki, EE., Tura,c G., Yilmaz, I. and Karaoz, E. 2014. BMP-2,-6 and BMP-7 differently regulate osteogenic differentiation of human periodontal ligament stem cells (hPDLSCs). *J Biomed Mater Res B.,* 2:119–30.

Hakki, SS., Kayis, SA., Hakki, EE., Bozkurt, SB., Duruksu, G., Unal, ZS., Turaç, G. and Karaoz, E. 2015. Comparison of mesenchymal stem cells isolated from pulp and periodontal ligament. *J Periodontol.,* 86:283–91.

Hiremath, H., Saikalyan, S., Kulkarni, SS. and Hiremath, V. 2012. Second-generation platelet concentrate (PRF) as a pulpotomy medicament in a permanent molar with pulpitis: a case report. *Int Endod J.,* 45:105-12.

Howard, D., Buttery, LD., Shakesheff, KM. and Roberts, SJ. 2008. Tissue engineering: strategies, stem cells and scaffolds. *J Anat.,* 213:66-72.

Huang, FM., Yang, SF., Zhao, JH. and Chang, YC. 2010. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. *J Endod.,* 36:1628-32.

Huang, GT., Gronthos, S. and Shi, S. 2009. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res.,* 88:792–806.

Jayalakshmi, KB., Agarwal, S., Singh, MP., Vishwanath, BT., Krishna, A. and Agrawal, R. 2012. Platelet-rich fibrin with beta-tricalcium phosphate-a noval approach for bone augmentation in chronic periapical lesion: a case report. *Case Rep Dent.,* 2012; 902858.

Keswani, D. and Pandey, RK. 2013. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. *Int Endod J*., 46:1096-104.

Kevy, SV. and Jacobson, MS. 2004. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol.,* 36:28-35.

Knighton, DR., Ciresi, KF., Fiegel, VD., Austin, LL. and Butler, EL. 1986. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann Surg.,* 204(3):322-30.

Knighton, DR., Doucette, M., Fiegel, VD., Ciresi, K., Butler, E. and Austin, L. 1988. The use of platelet derived wound healing formula in human clinical trials. *Prog Clin Biol Res.,* 266:319-29.

Lozada, JL., Caplanis, N., Proussaefs, P., Willardsen, J. and Kammeyer, G. 2001. Plateletrich plasma application in sinus graft surgery: Part I--background and processing techniques. *J Oral Implantol.,* 27:38-42.

Marlovits, S., Mousavi, M., Gabler, C., Erdös, J., Vécsei, V., *et al*., A new simplified technique for producing platelet-rich plasma: A short technical note. *Eur Spine J.,* 13:102-6.

Marx, RE. 2001. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent.,* 10:225-8.

Mehta, S. and Watson, JT. 2008. Platelet rich concentrate: basic science and current clinical applications. *J Orthop Trauma.,* 22:432-8.

Platelet Rich Plasma (PRP) Guidelines, © International Cellular Medicine Society-2011. Available from: http://www.cellmedicinesociety.org.

Schliephake, H. 2002. Bone growth factors in maxillofacial skeletal reconstruction. *Int J Oral MaxillofacSurg*., 31:469-84.

Shivashankar, VY., Johns, DA., Vidyanath, S. and Sam, G. 2013. Combination of platelet rich fibrin, hydroxyapatite and PRF membrane in the management of large inflammatory periapical lesion. *J Conserv Dent.,* 16: 261-4.

Sunitha Raja, V. and Munirathnam Naidu, E. 2008. Platelet-rich fibrin: Evolution of a second-generation platelet concentrate. *Indian J Dent Res.,* 19:42-6.

Sweeny, J. and Grossman, BJ. 2002. Blood collection, storage and component preparation methods. In: Brecher M, editor. Technical Manual. 14th ed. Bethesda MD: *American Association of Blood Banks (AABB),* 955-8.

Toffler, M., Toscano, N., Holtzclaw, D., Corso, MD. and Dohan Ehrenfest, DM. 2009. Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. *J Implant AdvClin Dent.,* 1:21-30.

Waters, JH. and Roberts, KC. 2004. Database review of possible factors infuencing point-of-care platelet gel manufacture. *J Extra CorporTechnol.,* 36:250-4.

Welsh, WJ. 2000. Autologous platelet gel: Clinical function and usage in plastic surgery. *Cosmetic Derm.,* 11:13-9.

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