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RESEARCH ARTICLE

CLINICAL AND HISTOLOGICAL EVALUATION OF L-PRF AND T-PRF – A COMPARATIVE REVIEW

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ABSTRACT

Wound closure and healing is the prime goal of a surgeon. With modern advances and techniques, therapeutic application of growth factors from autologous blood has demonstrated enhanced healing properties. Platelet rich products like second generation (L-PRF) and third generation (T-PRF) platelet concentrates have simplified the process without biochemical handling of blood. But, recent researches have raised controversies over the use of glass tubes for centrifugation of platelet concentrates like L-PRF as the silica contained in the end product may prove cytotoxic to human cell thus, in an attempt to eliminate all the possible risks, a more biocompatible platelet concentrate (T-PRF) was developed which used titanium tubes for centrifugation. T-PRF not only proved safe but also showed improved tissue healing and better activation of platelets and growth factors at the surgical site.

Study design: The purpose of this review was to compare the healing properties of T-PRF over L-PRF and explore its possible applications in the field of oral and maxillofacial surgery.

Result: T-PRF proved similar to L-PRF in terms of the clot produced which was clinically identical to each other but showed different healing properties and histocompatibilities. With stronger fibrin network, better biocompatibility and advanced resorption characteristics, T-PRF proved more promising in wound healing, angiogenesis and graft handling properties as compared to L-PRF.

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INTRODUCTION

Evidence suggests that blood derived products were used to seal wounds and stimulate healing with the use of fibrin glue which was invented 40 years ago (Ehrenfest *et al.,* 2009; Tunalı *et al.,* 2014). Many advances have been reported over the years to improve healing and replace fibrin glue, since first described by Whitman *et al.* 1997; Choukroun *et al.* in 2001 developed an autologous biomaterial that contains leucocytes and PRF. Platelet concentrates like PRF were originally used for prevention and treatment of haemorrhages during surgeries consisting of $0.5*10^{11}$ platelets per unit. PRF has ever since gained popularity as it is neither technique sensitive nor requires any anticoagulants or bovine thrombin (Tunalı *et al.,* 2013). But clinicians like O'Connell have reported possible health hazards of using glass-evacuated collection tubes for the blood with silica activators. It was reported that the use of glass tubes for centrifugation and storage of the fibrin product showed noncompliance to ISO 10993. He reported that although the silica particles are dense enough to sediment with red blood cells; a small fraction of colloidal suspension remains in the buffy coat with fibrin and platelet plasma layers thus, contaminating any therapeutic application to the patient. It also has an open system architecture which is a potential for microbial and chemical contamination of the materials before application to the patient's wound site thus, proving cytotoxic on a wide range of human cells[4]. A new platelet concentrate T-PRF (Titanium PRF) was prepared which was based on the hypothesis that titanium tubes may be more effective at activating platelets than glass tubes used in Chouckroun's method (Tunalı *et al.,* 2014; Tunalı *et al.,* 2013). Titanium in T-PRF not only helps in activating platelets better than silica but also shows better biocompatibility (Tunalı *et al.,* 2013). The objective of this review is to compare the clinical and histopathological effects of T-PRF and L-PRF in oral and maxillofacial surgery and also an attempt to address the controversies with an effective solution to the potential cytotoxic nature of L-PRF.

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MATERIALS AND METHODS

Structured electronic and manual search of scientific papers was conducted in Pubmed, Google Scholar and major journals published from 1954 to 2017. Abstracts of all relevant studies were thoroughly scrutinized and articles pertaining to the topic PRF were included.

Inclusion criteria: All case reports and series, original research papers, review papers, animal studies and controlled clinical trials on PRF used in dentistry.

Exclusion criteria: Studies that did not meet the above inclusion criteria. This article discusses history, types of PRF, techniques involved, advantages, disadvantages and limitations of various clinical research papers and studies on L-PRF and T-PRF and also explores its applications in Oral and Maxillofacial surgery.

History: The evolution of Platelet concentrates over the years and its applications in various fields of medicine and surgery has been remarkable (Table 1) (Agrawal *et al.,* 2017).

What is PRF?

Platelets consist of growth factors such as PDGF- AB (platelet derived growth factor AB), TGF beta-1 (transforming growth factor beta-1) and VEGF (vascular endothelial growth factor) which stimulate cell proliferation, matrix remodelling and angiogenesis. Fibrinogen activates to form a plasmatic molecule of fibrin. This is a soluble molecule, massively present in plasma and platelet alpha granules helping in platelet aggregation and hemostasis. It has the capability to transform into biologic glue and helps in forming a protective wall along vascular breaches during coagulation. Being the final substrate of all coagulation reactions, it transforms into an insoluble fibrin by thrombin while the polymerized fibrin gel remains as the first cicatricial matrix of the injured site (Clark, 2001; Van Hinsbergh *et al.,* 2001).

Advantages of PRF over PRP (Chhabra *et al.,* 2013; McAleer *et al.,* 2006): Simplified and cost-effective process which does not require bovine thrombin and anticoagulants. It demonstrates favourable healing due to slow polymerization, more efficient cell migration and proliferation. PRF also has supportive effect on immune system and primarily helps in haemostasis.

Types of PRF: Subfamilies of Platelet-Rich Fibrin (PRF) have varied composition, advantages and disadvantages (Table 2).

Need for T-PRF (Titanium prepared PRF) – An Improved Platelet concentrate: In 2013- 2014 a new breakthrough was achieved when Tunali *et al.* (Del Corso *et al.,* 2010; Tunalı *et al.,* 2014; Reddy *et al.*) discovered Titanium prepared Platelet-rich fibrin (T-PRF); a third generation platelet concentrate to overcome the hazardous effects of silica in the glass tubes which were previously used for PRF preparation (Tunalıq *et al.,* 2014; O'Connell, 2007; Matras *et al.,* 1985; Ustaoğlu *et al.,* 2016). Literature suggests that histologic analysis of T-PRF showed better polymerized fibrin formation with a longer resorption rate in the tissues as titanium was more effective in activating platelets than the silica activators in glass tubes (Tunalı *et al.,* 2014 & 2013). This has shown to increase the duration of release of growth factors with that of PRF, attributing to the thicker fibrin meshwork (Reddy; Tunali *et al.,* 2016; Naik *et al.,* 2013). Thus it was concluded that T-PRF can be used in conjunction with bone grafts, which offers several advantages including promoting wound healing, bone growth and maturation, graft stabilization, hemostasis and improving the handling properties of graft materials (Tunalı *et al.,* 2014; Matras, 1985; Ustaoğlu *et al.,* 2016; Tunali *et al.,* 2016; Naik *et al.,* 2013; Agrawal, 2017; Simonpieri *et al.,* 2012; Del Corso *et al.,* 2012).

***Preparation of L-PRF and T-PRF* (Huang *et al.,* 2017):**

L-PRF: Second generation platelet concentrate, obtained by natural process without any anticoagulants or jellifying agents. Venous blood is collected and centrifuged at low speed yielding RBC layer, PRF clot in middle and acellular plasma top layer. Approximately, 9mL glass coated plastic tube is centrifuged at room temperature at 2700 rpm (around 400 g) for 12 min.

T-PRF: Third generation platelet concentrate; Titanium tubes are used for collection and centrifugation instead of glass tubes. Intravenous blood is collected in a 10 ml sterile titanium test tube without anticoagulant. The tubes are immediately centrifuged at 3000 rpm for 10 minutes in a centrifuge machine. Blood centrifugation immediately after collection allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and a cellular plasma (Platelet Poor Plasma (PPP) at the top.

DISCUSSION

Autologous blood has been successfully used in applications of head and neck surgery. In oral and maxillofacial surgery, platelet concentrates have been successfully used in conjugation with ablative surgical procedures of the maxillofacial regions, mandibular reconstruction, and surgical repair of alveolar clefts and oro-antral fistulas (Whitman *et al.,* 1997). The concentration of parameters doesn't seem to be very crucial when it comes to oral and maxillofacial surgeries, as they are generally used after activation into gel and are placed in an open surgical site with blood, edema and collected fluids which may dilute the impact of platelet concentrates. It is the fibrin architecture and leukocyte content which is of utmost importance (Simonpieri *et al.,* 2012; Del Corso *et al.,* 2012). L-PRF has been widely used over the years with success but the discovery of potential risk and cytotoxicity associated with the silica contained in the end product due to glass tube centrifugation process (O'Connell *et al.,* 2007) led to the discovery of T-PRF[3]. It is a platelet leukocyte rich fibrin similar to that obtained from the classical L-PRF method, but the usage of titanium tubes in the centrifugation makes it safer and biocompatible. Titanium is one of the corrosion resistant materials which forms an adhesive oxide layer and becomes passive in vitro, creating an excellent functional network with the underlying bone and exhibiting osseointegration (Takemoto *et al.,* 2004). Hemocompatability of titanium makes it suitable for biomedical devices and grafts used in surgeries (Ratner *et al.,* 2004). Human trials comparing L-PRF and T-PRF confirmed that the fibrin network created with T-PRF was more tightly woven and thicker because titanium helped in formation of better polymerized fibrin structure as compared to the silica present in L-PRF which interfered with the polymerization procedure (Tunalı *et al.,* 2014). Animal studies demonstrated that increased duration and speed of centrifugation helped in the formation of clinically mature T-PRF clots (later termed as MT-PRF) which initiated better healing and superior bone formation as compared to L-PRF (Tunalı *et al.,* 2014). T-PRF shows increased duration of release of growth factors as compared to L-PRF, due to its stronger fibrin network and longer resorption rate in the tissues, can be used in conjugation with bone grafts as it offers excellent wound healing, bone growth, haemostasis and better handling of graft materials (Tunalı *et al.,* 2014; Reddy; Tunali *et al.,* 2016).

Table 1. History of platelet concentrates and its evolution

Serial number	Researcher	Year	Contribution
1	Kingsley	1954	First used the term PRP to describe thrombocyte concentrate during experiments related to blood coagulation.
2	Matras	1970	"Fibrin glue" was introduced which improved healing of skin wounds which was made by polymerizing fibrinogen with thrombin and calcium. *Limitation*: Low concentration of fibrinogen in donor plasma; the quality and stability of fibrin glue was suboptimal.
3	-	1975-1978	Numerous research works suggested an enhanced concept for using blood extracts and designated them as "platelet-fibrinogen-thrombin mixtures".
4	-	1979	Another author called it "gelatin platelet - gel foam". This new proposition asserted the performance of platelets, and demonstrated exquisite preliminary results in general surgery, neurosurgery and ophthalmology.
5	Knighton *et al*	1986	First demonstrated that Platelet concentrate successfully promotes healing and they termed it as "platelet-derived wound healing factors (PDWHF)".
6	Kingsley et al and Knighton et al	1988, 1990	Renamed the concentrate as "platelet-derived wound healing formula (PDWHF)".
7	Whitman *et al*	1997	Named the product PRP and later renamed it as "platelet gel".
8	Marx *et al*	1998	Some commercial companies, in lieu of better visibility, started labelling their products with distinct commercial names.
9	-	1999	One of the popular methods advertised on large scale to prepare pure platelet rich plasma was commercialized as plasma rich in growth factors (PRGF) or also called as preperation rich in growth factors (Endoret, Victoria, Biotechnology Institute BTI, Spain). *Limitation:* Lack of specific pipetting steps and also lack of ergonomics, there were significant issues with this technique.
10	Choukroun *et al*	2000	Developed another form of Platelet concentrate in France which was labelled as PRF, based on the strong fibrin gel polymerization found in this preparation. It was stamped as a "second-generation" Platelet concentrate. This proved an important milestone in the evolution of terminology.
11	Bielecki et al and Cieslik-Bielecka *et al*	2006	Defined PRP as inactive substance, and suggested PRG (Platelet Rich Gel) as a more biologically activated fibrin matrix rich in platelets, leukocytes and relative active molecule.
12	Sacco	2006	Introduced a new concept of CGF (concentrated growth factors). For making CGF from venous blood, rpm in range of 2400-2700 was used to separate cells.
13	Everts *et al*	2008	Focused on the leukocyte component of the platelet concentrate and the two forms, i.e., nonactivated and activated. The inactivated/non-activated product was called "platelet-leukocyte rich plasma (P-LRP) and activated gel was labelled platelet-leukocyte-gel" (PLG).
14	Dohan Ehrenfest *et al.*	2009	First classified the platelet concentrates. This classification defined 4 main families: (1) Pure platelet-rich plasma (P-PRP); (2) Leukocyte-and platelet-rich plasma (L-PRP); (3) Pure PRF (P-PRF); and (4) Leukocyte- and platelet-rich fibrin (L-PRF).
15	Sohn	2010	Conceptualised sticky bone (autologous fibrin glue mixed with bone graft).
16	Mishra *et al*	2012	Proposed a classification which was limited to PRP and applicable to sports medicine only. They stated 4 types of PRP based on presence or absence of leukocytes and whether or not the PRP is activated and all types can fall into 2 sub-types: A: Platelets > 5 × baseline or B: Platelets < 5 × baseline.
17	DeLong *et al*	2012	Introduced another classification system called PAW (Platelets quantity, Activation mode, White cells presence). *Limitation:* However it was only restricted to PRP families and was similar to classification by Mishra et al.
18	Choukroun	2014	An advanced PRF called APRF was developed.
	Tunalı *et al*	2013-2014	Introduced a new product called T-PRF (Titanium prepared PRF).
19	Mourão et *al*	2015	Gave detailed technical note on preparation Of I-PRF (Injectable PRF).

Table 2: Classification of PRF

Serial number	Type	Contents	Advantages	Disadvantages
1	P-PRF (Pure PRF)	Platelets ($400*10^3$ μL^{-1}) with high density fibrin network which is with minimal leucocytes ($0.1\text{-}0.6*10^3$ μL^{-1}).	Only exists as gel after activation.	Solid gel form; cannot be injected.
2	L-PRF (Leukocyte PRF)	Platelets ($400*10^4$ μL^{-1}) with high density fibrin network primarily composed of leucocytes ($60*10^3$ μL^{-1}).	Gel form; without any anticoagulant or natural blood clot.	Solid gel form; cannot be injected.
3	T-PRF (Titanium prepared PRF)	Same as L-PRF but without silica; contains titanium as a trace element.	Biocompatible; stronger fibrin network; better osteogenic potential.	High cost of preparation with titanium tubes, in comparison with glass tubes.
4	A-PRF (Advanced PRF)	CD3-, CD20-, CD34-, and CD68-positive cells near the BC (ie, the very proximal part of the fibrin clot)	Earlier vascularization, faster soft tissue growth, more cytokines and release of BMPs.	Prepared in glass tubes; contains silica in the prepared concentrate.
5	I-PRF (Injectable PRF)	Thrombocytes, leucocytes, monocytes, neutrophils and stem cells.	Liquid form; injectable.	Technique sensitive; risk of allergies or discomfort at the site of injection.
6	S-PRF (Standard PRF)	Tlymphocytes (CD3-positive cells), B-lymphocytes (CD20-positive cells), stem cells (CD34-positive cells), and monocytes (CD68-positive cells	Less technique sensitive.	Slower activation of platelet concentrate and growth factor; depth penetration of monocytes is poor.

T-PRF is shown to upgrade phosphorylated extracellular signal regulated protein kinase expression and suppression of osteoclasts by promoting the secretion of osteoprotegenin in osteoblastic cultures. It is stimulates osteogenic potential by differentiation of human dental pulp cells by upgrading the alkaline phosphatases (Saluja *et al.,* 2011). Healing with T-PRF is shown to increase tissue thickness and resemble primary healing rather than secondary healing thus, having an excellent soft tissue closure due to stronger cellular matrix which promotes cell migration and growth factor release (Dohan Ehrenfest *et al.,* 2010). Literature available on T-PRF and L-PRF is very limited and complex unlike PRP. T-PRF and L-PRF regulate the interaction of soft and hard tissues in order to promote synchronized healing but titanium present in T-PRF creates a more homogenous environment with stronger tissue matrix and improves osseointegration, bone regeneration and dcreases periimplantitis and bone defects. Thus, the maturation and healing potential depends not only on the product but also on the centrifugation time and process (Tunali *et al.,* 2016) but this theory lacks evidence and requires further evaluation.

Limitations (Arora *et al.,* 2009)

Low platelet counts and use of antiplatelet drugs can be a limiting factor and might hinder the efficacy; patients with known thrombotic risk factors might be under risk while undergoing treatment with Platelet concentrate and being an autologous blood source it is available in limited quantities only.

Conclusion

Blood handling in titanium tubes causes massive activation of collected platelets with release of the many cytokines unlike glass tubes where the meshwork is less dense and contains silica particles (Tunalı *et al.,* 2014; Dohan *et al.,* 2006), some studies suggest that these soluble molecules might get partially trapped in the fibrin meshes of the PRF and there is still no comparative quantification in support of better healing potential with T-PRF over L-PRF. The authors believe that this article will help to look further into PRF associated biologic mechanisms, help create better correlation with clinical results and foresee new prospects for the use of this promising biomaterial.

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