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RESEARCH ARTICLE

PLATELET CONCENTRATE APPLICATION IN COMBINATION WITH BONE GRAFT IN ODONTOGENIC CYST

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ABSTRACT

The use of platelet concentrate in dentistry is contemporary and revolutionary for the tissue regeneration. Platelet concentrate carries cells, growth factors and some cytokines which is becoming a valuable adjunct to promote natural healing in many procedures in periodontal surgery. This is the goal in periodontal regenerative surgery that is to achieve complete wound healing and regeneration of the periodontal unit. Platelet concentrate in the form of Platelet Rich Plasma (PRP) was first to be introduced and being extensively used for regeneration. Recent development of Platelet Rich Fibrin (PRF) by Choukroun revolutionaries its use in surgical procedure because of ease of preparation and increase amount of platelet with active growth factors available is more than PRP. It remains controversial because of its duration of growth factor release. Within last 2-3 years there are research being attempted to increase the amount of platelets in fibrin mesh by Shahram Ghannati *et al.* and increasing the fibrin polymerization to incorporate platelet for more duration by Mustafa Tunali *et al*. This article is being attempted to review past and present development of platelet concentrate and its effect in odontogenic cyst with bone graft.

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INTRODUCTION

There has always been a search for new materials and technologies to enhance regenerative process of human body. Utilizing the patient's own blood is a unique concept in dentistry. The healing of hard and soft tissue is mediated by a wide range of intra and extracellular events that are regulated by signaling proteins. So using those proteins or cells which releases these proteins will be ideal for regeneration and platelet is one of them. The platelet was discovered and termed by James Wright in 1880[1]. It was seen that the platelets not only had haemostasis property but also had a part to play in the wound healing process. This mainly is due to the growth factors released from the platelets[2-5]. Growth factors are the biologically active substance that is involved in tissue repair mechanism such as chemotaxis, proliferation, angiogenesis, extracellular matrix deposition and remodelling. Alpha granules are storage units within platelets which contain prepackaged growth factors in an inactive form. The main growth factors are Platelet- derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin like growth factors (ILGF), transforming growth factor β (TNF-β), fibroblast growth factor (FGF) and epidermal growth factor (EGF) [6,7].

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(Table 1) This led to the use of platelet concentrate in regenerative medicine and dentistry. Platelet concentrate are defined as autogenous products prepared through centrifugation of blood sample of the patient. Numerous techniques of autologous platelet concentrates have been developed[17]. The first generation incorporates the platelet rich plasma which was introduced by Marx *et al.*[19] in 1998 while the second generation involves platelet rich fibrin which was discovered by Choukroun[20]. Thus, platelet rich concentrate has been used as a method of introducing concentrate growth factors to the surgical site thereby enhancing the natural blood clot to hasten wound healing and stimulate bone regeneration.

Platelet Rich Plasma

Platelet Rich Plasma (PRP) is considered 1st generation platelet concentrate. A PRP blood clot would contain 4 percent RBC, 95 percent platelet and 1 percent WBCs compared to blood clot containing 95 percent RBC, 5 percent platelet and less than 1 percent WBCs[21]. The basic PRP protocol requires collection of blood with anticoagulant, centrifugation (either one spin or two spin) with $CaCl_2$ and bovine thrombin to induce polymerization of fibrin[19]. There are multiple variations on the major techniques used to concentrate autologous platelets from whole blood[17]. Even though, all involve centrifuging whole blood to concentrate platelets. These preparations varies on the basis of

1. Number and speed of centrifuge spin
2. Amount of blood required
3. Collection of platelets with or without leukocyte
4. Means of platelet activation
5. Type of instrument required
6. Use of purchased kit

methods of PRP preparation along with their advantage and disadvantage.

Thus, Dohan Ehrenfest *et al.* [30] have classified these preparations into four main families of preparations depending on their cell content and fibrin architecture.

Table 1. Platelet containing Growth Factors and their function

Cell	Cytokines	Function
Platelets	Platelet- derived growth factor (PDGF)	• Migration, proliferation and survival of mesenchymal cells in each stage of wound healing[8-10] • Stimulate macrophages to synthesize and secrete TNF-α & β[11] • Helps in angiogenesis[8,9]
	Vascular endothelial growth factor (VEGF)	• Initiate angiogenesis[11,12]
	Insulin like growth factors (ILGF)	• Have chemotactic effect towards osteoblasts and its proliferation[13] • Enhance wound healing[14-16]
	Transforming growth factor β (TNF-β)	• Act as paracrine growth factor and stimulate preosteoblast and formation of woven bone.[13] • Also stimulates angiogenesis
	Fibroblast growth factor (FGF)	• Stimulates osteoblasts proliferation[15] • Enhance wound healing with VEGF[17]
	Epidermal growth factor (EGF)	• Stimulates cell proliferation (increases tensile strength of the wound) and extracellular matrix turnover[11,18] • Chemotactic effect on fibroblast cells[11]

Table 2. Different methods of Platelet Concentrates and their method of preparation

Types	Method	Advantage/ Disadvantage
A. Cell separator[17]	Used in blood banks to separate	• Separated by large complicated and expensive automated machine • Large amount of blood is required (~120ml) • Loss of large amount of activated platelets
B. Classical PRP (Harvest smart PReP,[25] Curasan,[24] Ace systems,[22] Friadent-Schitze,[26] Reyen,[27] Plateltex[28])	• 2 spin method • Only PPP suspension is taken in 2nd spin • In Curasan 2400rpm for 10 min in 1st spin and 3600rpm for 15 min in 2nd spin.	• Complicated method • Provide leukocyte rich, platelet rich plasma
C. Platelet Rich in Growth Factor (PRGF) (Anitua,[23] Nahita[22])	• Slow speed Single spin (2000 rpmfor 7 min)	• Technically easy to prepare • Leukocyte poor and platelet poor concentrate preparation
D. Platelet Rich Fibrin Matrix (PRFM) (Fibrinet PRFM[25])	• 2 spin method • 2nd spin use $CaCl_2$ to form fibrin matrix • 2400rpm for 10 min in 1st spin and 3600rpm for 15 min in 2nd spin	• Lower platelet yield because of loss of platelet due to longer time of 2nd spin
E. Simplified Buffy Coat -PRP Simplified BC- PRP (Rutkowski 2008[29])	• Single high spin • Use of PRP and buffy zone of platelet concentrate • PRP only 3mm above and 2mm bellow the buffy coat	• Consist of buffy zone which contains maximum leukocyte • PRP zone consist of maximum amount of active platelet.

Table 3. Leukocyte function in PRF

Cell	Cytokines	Function
Leukocyte	Serine[33]	• Primary role is to phagocytize debris, microbes and necrotic tissue to clean wound and prevent infection[32] • Platelet and lymphocytes activation • Antimicrobial action • Cytokines activation and inactivation • Formation of fibrin- platelet plug
	MMPs[33,37,38]	• Degrade fibrin- platelet clot and remodel the extracellular matrix which helps in release of growth factors • Controls inflammatory response • Helps in PMNs migration,angiogenesis, re-epithelization and tissue remodelling.[37,38]

The variations in PRP preparations result in compounds with significantly dissimilar growth factor and platelet concentration as well as biomechanical properties of the matrix. Specific protocols and automated systems for preparing PRP have been developed and commercialized. These include Ace,[22] Anitua,[23] Nahita,[22] Curasan,[24] PCCS,[25] Harvest Smart PReP,[25] Vivostat,[25]Friadent-Schitze,[26] Reyen,[27] Fibrinet PRFM,[25] Plateltex[28] and they all give products which are slightly differs histologically. Thus the Table 2 summarizes the different

1. Pure platelet-rich plasma (P-PRP)
2. Leukocyte and platelet-rich plasma (L-PRP)
3. Pure platelet-rich fibrin (P-PRF)
4. Leukocyte and platelet-rich fibrin (L-PRF)

These are liquid platelet concentrate suspensions (before activation), whereas, solid platelet with strong fibrin architecture are termed Platelet Rich Fibrin (PRF). Presence of leukocyte was considered important to classify platelet concentrate because it have key role in wound healing.[31-36] The

PMNs are first to migrate towards wound site and have important role in clearing wound. It also releases some proteinases, such as Serine and Metalloproteinase (MMP),[33] which have critical role in wound healing (Table 3).

Platelet Rich Fibrin

PRF is considered 2nd generation platelet concentrate developed in France by Choukroun *et al.* [8,20] in 2001. It consists of platelets, cytokines and fibrin matrix. Unlike PRP this technique does not require anticoagulants, bovine thrombin and other gelifying agent. The PRF clot is yielded by a natural polymerization process during centrifugation and its natural fibrin structure seems responsible for a slow release of growth factor, matrix glycoproteins during ≥ 7 days.[39] The fibrin meshwork in PRF is flexible, elastic and very strong. Whereas in PRP there are bilateral junctions that doesn't honor the cytokine enmeshment and cellular migration. This is an advantage over PRP where active growth factor secretion is initiated as soon as clotting process begins. Within 10 minutes after clotting more than 95 percent of presynthesized growth factors are released within 1 hour.[40] That's why it is considered to use these preparations within 10 minutes of clot initiation in both PRP and PRF. PRF has many other advantages over PRP like there is no use of bovine thrombin and anticoagulant hence no antibody reaction which may result in life threatening coagulopathies in some individuals. It is simplified because there is no biochemical handling required and doesn't require specific instrumentation as that in PRP, so it is cost effective. More efficient cell migration and proliferation is seen in PRF because of its fibrin mesh[41].

PRF is prepared by drawing 10 ml blood and placing it into the test tube, then centrifuged immediately for 12 min at 2700 rpm (Some authors have used 3000 rpm for 10 min).[8] Platelet coming in contact with glass test tube will activate fibrin polymerization. Later it was found that it incorporates some leukocytes with platelets and considered L-PRF according to Dohan *et al*. classification.[30] Recently, advances in L-PRF method of preparation resulted in Titanium- Platelet Rich Fibrin (T-PRF), Advanced- Platelet Rich Fibrin (A-PRF) and Injectable- Platelet Rich Fibrin (I-PRF). Mustafa Tunali *et al*. (2013) used titanium coated glass test tube instead of glass test tube. Centrifugation cycle and duration was similar to the L-PRF and he found fibrin formed was more tightly woven than L-PRF. He postulated that the T-PRF may last longer and release growth factors slowly.[42] Shahram Ghannati *et al*. (2014) and used 1500 rpm for 14 min instead of 2700 rpm for 12 min and he found even distribution of platelet in fibrin clot with excess of leukocyte in comparison to L-PRF. He termed it Advanced PRF.[43] Recently, Choukroun and Antoine Blomart (2014) introduced I-PRF similar to injectable PRP and being marketed by Process For PRF®. They used 700 rpm for 3 min and it allows polymerization of fibrin after centrifugation, so it can be easily mixed with bone graft (form fibrin matrix incorporated with bone graft). This will help in carrying the bone graft to defect site.

Clinical Application of L-PRF

L-PRF are applicable in accelerating the healing in sinus lift procedures, socket preservation, ridge augmentation, infrabony or osseous defect, jaw reconstruction and soft tissue procedures like root coverage procedure, gingival graft and subepithelial graft. Many studies have been done to reconstruct osseous defect with L-PRF, without bone graft.[44-46] Kleinheinz *et al*.[47] (2003) compared the effects of Platelet concentrate on osteoblasts and endothelial cells and found out that the activation of endothelial cells leads to increased angiogenesis and thus indirectly to increased osteogenesis. It is questionable, in large osseous defect like cystic defect, socket preservation and sinus lift, that if an increased concentration of thrombocyte-associated factors will eventually lead to improved bone regeneration or shorten the healing period.[48] So, in these conditions bone graft can be added. Many previous studies have shown clinical success in bone formation with the use of L-PRF and bone grafts material separately. So, the use of L-PRF with bone graft can give added effect of both. In present case report an innovative idea of combination of L-PRF membrane with beta-tricalcium phosphate (β-TCP) was used.

Case Report

A 25 year old male visited the Department of Prosthodontics, JSS Dental College and Hospital with the chief complaint of broken fixed partial denture since 6 month. He had history of using fixed partial denture for 8 years. No relevant medical history was noted. On clinical examination maxillary right central incisor 11 was missing with history of trauma 8 years back. Maxillary left central incisor 21 was root canal treated with fractured crown. Duration of fractured crown was 6 month. On routine radiographic examination, a round radiolucency with radiopaque margin was seen in relation to 21 (Fig.1). Provisional diagnosis of residual periapical cyst was made and referred to Department of Conservative Dentistry. Re-endodontic therapy was planned. After re-endodontic treatment patient was referred to Department of Periodontology for surgery.

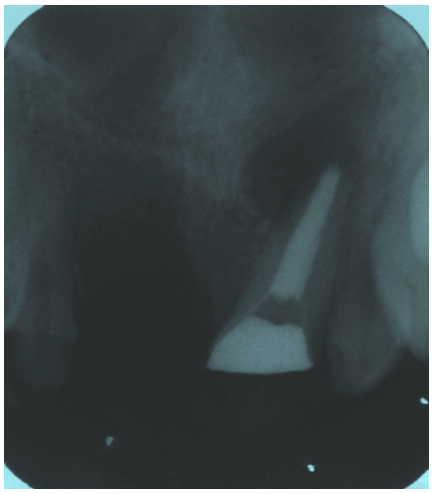

Fig. 1. Note the radiolucent area at the apices with a diameter of larger than 0.8 cm

After phase I therapy and complete blood examination patient was prepared for surgical treatment. Infra-orbital Nerve and Nasopalatine Nerve block was given with 2% Lidocaine with 1:80000 adrenaline local anesthesia (0.9ml and 0.5ml respectively). Full thickness flap was raised with two vertical

relieving incisions from right lateral incisor to left canine. On reflection it was seen that 0.5 to 0.8 mm cystic defect was present at the mesial surface of apical third of 21, suggestive of a lateral periodontal cyst. The area was thoroughly debrided and curettage done. Defect was filled with β-TCP bone graft (Septodont RTR cone®) and L-PRF membrane was placed over it. Flap was approximated with interrupted non-resorbable 4-0 silk suture and post-operative instructions were given. Patient was prescribed amoxicillin 500mg + clavulanic acid 125mg prophylactic antibiotic bid for 5 days and diclofenac sodium analgesic for 5 days. Patient was recalled after 1 week for suture removal and surgical healing was found to be satisfactory. Case was followed up at 6 (Fig.2) and 9 month (Fig.3) which showed adequate bone fill in intra oral periapical radiograph.

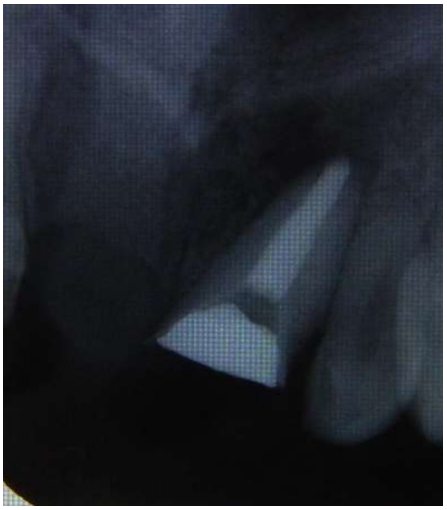

Fig. 2. After 6 month

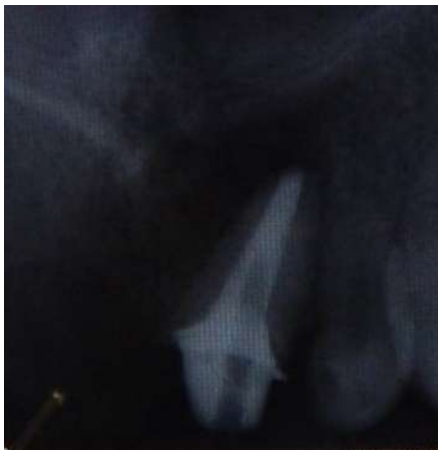

Fig. 3. After 9 month

DISCUSSION

Lateral periodontal cyst is an uncommon lesion of the oral cavity. It is an intraosseous (central) cyst, associated generally with root of vital tooth but the history of endodontic treatment in present case restricted to assess vitality. The lateral periodontal cyst occurs particularly between 5th through 7th decades, with an average of 54 years and it is uncommon in 2nd decade. Clinically it presents with no signs or symptoms as was in this case.[49,50] Correct diagnosis is of paramount importance, since misdiagnosis may lead to extraction of teeth. Recent year's use of PRF in dentistry sifted from repair to regenerative potential. Chang *et al* found out that PRF enhances the expression phosphorylated extracellular signal- regulated protein kinase (p-ERK), osteoprotegerin (OPG) and alkaline phosphate (ALP), which may enhances the expression of periodontal bone regeneration.[51,52] He also found beneficial effect of PRF in infrabony pocket on clinical and radiographic evaluation which was similar to the result obtained by Pradeep *et al*.[46]

Lee *et al.* compared autogenous grafts with autogenous grafts plus PRF for sinus lifting operations. In the histomorphometric examination, the amount of the bone in autogenous graft /PRF combination group was more than in the group treated with autogenous bone grafts alone. In this situation author found that PRF activates the protein structure in the autogenous grafts and osteoblasts tended to adhere on to the bone graft which indicates its own osteoinductive effect.[53] So if used with the bone graft substitutes will provide beneficial results. A clinical study was done by Pradeep *et al.* with Hydroxyapatite bone graft substitute and PRF in angular bone defect and found good result.[54]

Tricalcium phosphate as a bone graft substitute has been evaluated at length in many studies.[55] It binds to bone by means of mechanical anchorage with no formation of intermediate apatite layer. β-TCP material is slowly resorbed and replaced by natural bone which is facilitated by its porous structure. Bioresorption of β-TCP granules occurs due to chemical dissolution in biological fluids and cellular degradation.[56] Solubilization is induced by mesenchymal cells, which are also actively involved in the degradation process. Studies have shown the capability of osteoblastic cells, fibroblasts, and osteoclasts to degrade β-TCP ceramic material. Monocyte/macrophage participation is also well documented in vivo as well as in vitro.[57]

Histologic studies by Artzi Z *et al* have shown that β–TCP particles are resorbed in 12-24 months and replaced with the newly generated bone within 36 months.[58] This shows markedly slow resorption as required for the alloplast but it has 1/20th compressive strength of the cortical bone, so little swift transition to bone will be ideal with this bone graft. Similarly Wiltfang *et al.* compared the graft materials with Platelet concentrate in pigs and they observed β–TCP particles remained in the bone defects at 12th week in the prepared cavities with β–TCP alone but not in β–TCP with platelet concentrate.[59] It is thought that PRF accelerates the healing effect by keeping the particles of β-TCP together via its adhesive property and fastening the resorption process of graft substitute by cytokine like action on fibroblast and other cells.[59] As a result, it can be stated that PRF increases the transformation of β-TCP particles into bone.

Similarly Yazawa *et al.* studied Platelet concentrate, β– TCP and fibrin adhesive in rabbits and they concluded that the use of Platelet concentrate with synthetic graft materials is successful.[60] Kim *et al* found more rapid healing of PRF mixed with β–TCP in sinus lift and bone defect of study model than recombinant human bone morphogenic protein 2 mixed with

β–TCP or β–TCP alone.[61] Similar result was found by Jaylakshmi *et al* when same combination was used in periapical bone defect. Del Fabbro *et al.* concluded from their study that the combined use of PRF with graft materials in bone defects contributes to wound healing.[63]

Conclusion

Adding PRF to β–TCP was considered to reduce the time required to promote graft consolidation, maturation, and improved trabecular bone density. So, combined use of PRF and β-TCP for bone healing is a potential treatment alternative for faster healing than using these biomaterials alone. In future the use of injectable PRF with bone graft would facilitate a better alternative for carrying of bone graft and hasten wound healing procedure.

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