Platelet Concentrates

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Introduction

Platelets play an important role in hemostasis andwound healing. Platelet derived growth factors are well knownsource of healing cytokines. Various techniques ofautologous platelet concentrates have been developed andapplied in oral and maxillofacial surgery.

Platelets are non-nucleated cytoplasmic fragments derivedfrom bone marrow megakaryocytes and measure 2–3 µmin diameter. They contain many granules, lesser mitochondriaand two prominent membrane structures, one the surface connected canalicular system and the other is dense tubular system.

α- granulesare intracellular storage pool ofproteins important for wound healing which including platelet-derivedgrowth factor (PDGF), transforming growth factor (TGF-β),and insulin-like growth factor (IGF-I).After activation the α granulesfuse with the platelet cell membrane. Few secretory proteins are transformed to a bioactivestate. The active proteins are then secreted, allowingthem to bind to transmembrane receptors of the target cells.

Bound, intracellular signal proteins are activated resulting in the expression of a gene sequence thatdirects cellular proliferation, collagen synthesis, osteoidproduction etc.

Platelet concentrates were originallyused in transfusion medicine, for the treatment and prevention of haemorrhagedue to severe thrombopenia, which is often caused bymedullar aplasia, acute leukaemia or significant bloodloss during long-lasting surgery. The standard plateletconcentrate for transfusion has been named platelet-richplasma (PRP) and classically contains 0.5 X 10^{11} plateletsper unit.

The use of blood-derived products to seal wounds andstimulate healing started with the use of fibrin glues,which were first describedin 1970's and are constitutedof concentrated fibrinogen (polymerization induced bythrombin and calcium).They are used for topicalhemostasis and tissue sealing and as melting agents forparticulate bone substitutes. Risk of cross infection forcommercial adhesives (Tisseel, Baxter healthcare) led to the development of autologous fibrin sealants from thepatient's own plasma. However, their fabrication resultedin less reproducible or less satisfactory rheologic properties.Autologous fibrin glues were consideredthe best choice to avoid contamination risk, buttheir use remains very limited owing to the complexity andthe cost of their production protocols.

Consequently, the use of platelet concentrates toimprove healing and to replace fibrin glues, as firstdescribed by Whitman et alin 1997.

Aim:

Aim of this paper /poster is to review the history and various types of platelet concentrates and their uses in dentistry.

Classification of Platelet Concentrates

Platelet concentrates now can be broadly classified as first generation platelet concentrates and second generation platelet concentrates.

First Generation Platelet Concentrate

Leucocyte-poor or pure platelet-rich plasma (P-PRP): Pure platelet concentrates for topical use were first developedas an additional application of the classical transfusionplatelet units and were first reported for maxillofacialsurgery by Whitman et al in 1997.

two type of methods were employed for its production

- Automated protocols for P-PRP: plasmapheresis with a laboratory cell separator and Vivostat PRF: either in a discontinuous flow set up (inwhich the patient stays connected to the machine and theblood filtering continues until the desired quantity ofplatelets has been collected) or starting from a bag ofharvested blood with anticoagulant.
- The Vivostat PRF centrifuge (Vivolution, Denmark can be considered as an advanced cell separator, and it was originally designed to produce the Vivostat Fibrin sealant. The use of a specific preparation kit with this centrifuge allows the production of a leucocyte-poor platelet concentrate for surgical use. However, Vivostat PRF has been used in only a few published studies, and this system is cumbersome and very expensive for daily practice. Moreover, its platelet collection efficiency is rather low and platelets are damaged during theprocess.
- Manual protocols for modified P-PRP: Anitua's PRGF: One of the first platelet concentrate protocols (PRGF,which stands for either plasma rich in growth factorsor preparation rich in growth factors) wasdescribed in 1999 by Anitua and has been commercializedby BTI (BioTechnology Institute, Vitoria, Spain). In thisprotocol, venous blood is collected and centrifuged in severalsmall tubes to obtain the three typical layers: RBCs,'buffy coat' and acellular plasma. The upper part of theacellular plasma is called plasma poor in growth factors(PPGF) and is discarded from each tube by careful pipettingto avoid creating turbulences. The remaining plasmais termed PRGF and is collected with a pipette, using only'eyeballing' as a measuring tool.

Leucocyte- and platelet-rich plasma (L-PRP): The initial objective of developing alternative easy-tohandle methods was to make it possible to use platelet concentrates in daily practice without having the support of a transfusion laboratory. Without a cell separator, elimination of leucocytes becomes more difficult, and the resulting platelet concentrates therefore contain a high quantity of leucocytes, which were not initially desired.

- Automated protocols for L-PRP: SmartPReP, PCCS, GPS and Magellan: Automated systems for L-PRP have been developed in the form of PCCS (Platelet Concentrate Collection System) by 3I (Palm Beach Gardens, USA)in 2006and SmartPReP by Harvest Corp (Plymouth, USA). The centrifugesused have been

designed to take a customized collectionand centrifugation device, which consists of two connectedcompartments. In the PCCS method, citrated whole bloodis transferred into the first compartment and centrifugedbriefly to obtain the three layers (RBC, buffy coat, PPP).Then, by opening of a tubule and using air pressure, thesuperficial layers (i.e. PPP and buffy coat) are transferredto the second chamber and centrifuged again but for alonger period. Finally, using the same air pressure system,most of the PPP layer is transferred back into the firstcompartment and thus discarded.

- Manual protocols for L-PRP: Curasan, Friadent-Schu¨tze, Regen and Plateltex:Two similar protocols, each using a two-step centrifugation procedure, were marketed by Curasan (Kleinostheim, Germany)and Friadent-Schu¨ tze (Vienna, Austria) respectively.Each method follows the first principalstep described above and shown in Figure, in which a firstcentrifugation step separates the blood components intothree layers of RBCs, 'buffy coat' and PPP. The PPP andbuffy coat layers are then carefully collected, avoiding RBCcontamination, and transferred to another tube, wherethey are subjected to a second centrifugation step at highspeed, which separates the sample again into its components.After the second centrifugation step, most ofthe PPP layer is discarded using the 'eyeballing' method.The PRP concentrate obtained with this method is composedof a high quantity of platelets, leucocytes and circulatingfibrinogen, but it also contains residual RBCs. Theconcentrate is applied with bovine thrombin and calciumchloride.

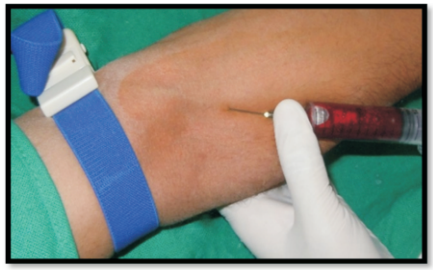
Leucocyte-poor or pure platelet-rich fibrin (P-PRF) concentrates: In this category, there is only one method available. TheFibrinet PRFM kit by Cascade Medical (New Jersey, USA) contains two tubes, one for blood collection and another forPRFM clotting, together with a transfer device. A smallamount of blood (typically 9 mL) is drawn into a collectiontube, which contains tri-sodium citrate as an anticoagulantand a proprietary separator gel, and centrifuged for sixminutes at high speed. The three typical layers of RBCs,buffy coat and PPP are obtained. Buffy coat and PPP areeasily transferred to a second tube containing CaCl2 withthe help of a specifically designed tube connection system.The clotting process is triggered by the presence of CaCl2 and the tube is immediately centrifuged for 15 min, after which a stable PRFM clot can be collected. The company claims that the system produces a 'natural' platelet concentrate owing to the absence of bovine thrombin. However, this claim is doubtful because the blood is mixed withanticoagulant and separation gel, leading to what could be considered unnatural conditions.

Advantages of Platelet Gel and PRP Over Fibrin Sealants Safe autogenous preparation, free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever and Creutzfeld–Jacob disease (mad cow disease).

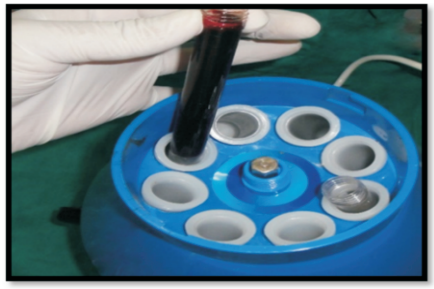
Convenient for patient since blood is collected in the immediate preoperative period.

More patients are eligible for this procedure because the criteria of blood bank donation do not have to be met, this would include children upto age 6, weights upto 25 kg, the elderly, those whose medical condition would preclude the blood bank from drawing a unit of whole blood.

Presence of platelets brings cytokines and growth factors to the site of surgery in a manner that would not occur with fibrin glue.



Withdrwal of blood

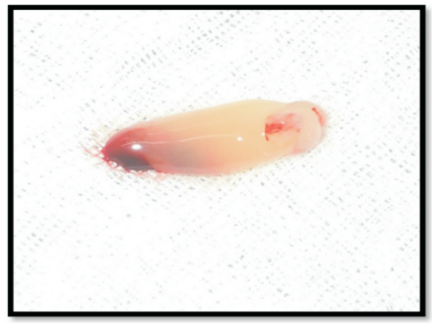


Centrifugation of blood

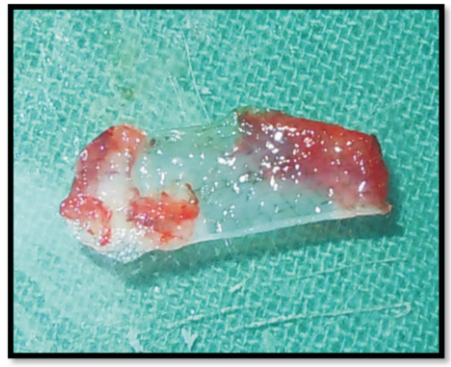
Centrifuged blood clot PRF clot

Second generation Platelet concentrate:

Leucocyte- and platelet-rich fibrin (L-PRF) concentrates: Choukroun's PRF:Choukroun's PRF protocol is a simple and free techniquedeveloped in France by Choukroun et al.. It can beconsidered as a second-generation platelet conc entrate because the natural concentrate is produced without anyanticoagulants or gelifying agents. Venous blood iscollected in dry glass tubes and centrifuged at low speed(Process protocol, Nice, France). In the absence ofanticoagulants, platelet activation and fibrin polymerization are triggered immediately. Therefore, after centrifugation, three layers are formed: the RBC base layer, acellular plasma top layer and a PRF clot in the middle(Figure 3). The PRF clot forms a strong fibrin matrix with aComplex three-dimensional architecture, in which most of the platelets and leucocytes from the harvested blood areconcentrated . When pressed between two gauzes, the PRF clot becomes a strong membrane, and some applications of this autologous biomaterial have been described in oral, maxillofacial, ENT (ear, nose, throat)and plastic surgery.



PRF clot is compressed between two glass slabs



PRF membrane

Figure : Preparation of **Choukroun's**PRF

Properties of PRF

(1) The biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on healing processes

(2) PRF is not only a platelet concentrate but also an immune node able to stimulate defense mechanisms. It is likely that the significant inflammatory regulation noted on surgical sites treated with PRF is the outcome of retro control effects from cytokines trapped in the fibrin network and released during the remodeling of this

initial matrix.

(3) Role of fibrin matrix of PRF:
Fibrin is the natural guide of angiogenesis.
Fibrin constitutes a natural support to immunity.
Fibrin and wound coverage: Fibrin matrix guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts

Clinical Implications of PRF

- In sinus lift procedures.
- Socket preservation.
- PRF membrane has been used for gingival recession coverage with coronally advanced or lateral pedicle flap for multiple and single recession respectively. PRF acts both as healing and interpositional biomaterial.
- Filling of cystic cavity.
- In the treatment of combined periodontic endodontic lesion/furcation defect.

Advantages of PRF over PRP

No need of addition of bovine thrombin or other anticoagulants so it is completely safe.
Standard production protocol.

Limitations of PRF Technology

(1) Only a limited volume of PRF can be used. Because itis obtained from an autologous blood sample, thequantities produced are low. This fact limits thesystematic utilization of PRF for general surgery.

(2) PRF tissue banks are unfeasible. The fibrin matrixcontains all the circulating immune cells and all thehighly antigenic plasmatic molecules. That is whyPRF membranes are totally specific to the donor andcannot constitute an allogenic graft tissue

Conclusion

Platelet concentrates are rich in growth factors. The role of growth factors in regeneration is undisputable, several in vitro and in vivo studies have provided evidence that the application of growth factors may increase tissue regeneration, Platelet rich and platelet-poor plasma have demonstrated results in the soft tissue regeneration, they might promote myofibroblastic differentiation, cell migration and extracellular matrixremodeling. but there are concerns weather these growth factors are available at the site for enough period of time?It is still uncertain which concentrations of platelet rich plasma are optimal for promoting wound healing also it was observed that the growth factors released by platelet concentrates gets washed away by GCF flow leaving very less or no available growth factors for regeneration. Hence further studies are required to acess the role of platelet concentrates in periodontal regeneration.

Difference Between first & second Generation Platelet Concentrates.[1]

First Generation-cPRP	Second Generation-PRP
Use of Bovine Thrombin & Calcium Chloride (Anticoagulants)	No Anticoagulants Used
Sudden Fibrin polymerization-depending on the amount of surgical additives (Thrombin & Calcium Chloride)	Slow Natural polymerization on contact with glass particles of the test tube results in physiologic thrombin concentration
3-D organization of a fibrin network-condensed tetra Molecular or bilateral Junctions constituted with strong thrombin concentrations, Allows the thickening of fibrin polymers: this leads to a rigid network, not very favorable to cytokine enmeshment & cellular Migration.	3-D network-connected trimolecular or equilateral junctions-allows the establishment of a fine & flexible fibrin network able to support cytokines enmeshment & cellular migration.
The 3-D Structure provides great resistance of such a gel, appropriate to firmly seal biologic tissues.	The 3-D Structure gives elasticity & flexibility to the PRF membrane.