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REVIEW ARTICLE

Platelet Rich Fibrin- Evolution, Preparation and its Clinical Implications

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***ABSTRACT*:**
Platelet Rich Fibrin, a second generation platelet concentrate is an autologous leukocyte, cellular and fibrin matrix. It consists of an assembly of cytokines, glycanic chains and structural gylcoprotein's enmeshed between a slowly polymerized fibrin network. It is free of any anticoagulant or other artificial biochemical modifications. It was first developed by Choukroun et al. (2001) The scientific rationale behind the use of platelet preparations lies in the fact that the platelet α- granules are a reservoir of many growth factors that play a crucial role in hard and soft tissue repair mechanism.
Key words: Platelet Rich Fibrin, growth factors, periodontitis.

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Platelets

Giulio Bizzozzero in 1882 discovered Platelets as a class of blood corpuscles.[1] These are anucleate cytoplasmic fragments 2–3 μm in diameter derived from bone marrow megakaryocytes.[2] The platelet's α granules (200 to 500 nm each) store platelet‐derived growth factor, transforming growth factor, and insulin‐like growth factor proteins essential for wound healing. The pioneers who first described a growth factor from platelets in 1974 were Ross et al.[3] Growth factors are proteins or polypeptides that leads to initiation of proliferation of cells that are in dormant state by stimulating DNA synthesis.[4] In dentistry the regenerative capacity of platelets was first familiarized in the 70s.

Evolution of Platelet Concentrates

1954

The term PRP was first used by Kingsley to allocate thrombocyte concentrate during blood coagulation related experiments.[5]

1970

Matras introduced "Fibrin glue" by showing improved healing of skin wounds in rat models. It was synthesized by polymerizing fibrinogen with thrombin and calcium. But in donor plasma concentration of fibrinogen was low, the fibrin glue quality and stability was suboptimal.[6]

1975-1978

Numerous investigators designated blood extracts as "platelet-fibrinogen-thrombin mixtures"[7]

1979

Some researcher named it "gelatin platelet - gel foam". This new term confirmed the action of platelets, and showed results in general surgery, neurosurgery and ophthalmology. But till then all these blood extracts were used for their "gluey effect", with no consideration of healing properties of growth factors.

1986

Knighton et al named platelet concentrate as "platelet-derived wound healing factors (PDWHF)".[8]

1988, 1990

Kingsley *et al* and Knighton *et al* used term "platelet-derived wound healing formula (PDWHF)".[5,9]

1997

Whitman *et al* named their product PRP but labeled it as "platelet gel".[10]

1998

The development of these techniques continued slowly until the article of Marx *et al*, which started the craze for these techniques. However, all these products were designated as PRP without deliberation of their content or architecture, and this paucity of terminology continued for many years. Some commercial companies, in lieu of better visibility, started labeling their products with distinct commercial names.[11]

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). However, because of lack of specific pipetting steps and also lack of ergonomics, there were significant issues with this technique. Another widely promoted technique for P-PRP was commercialized by the name Vivostat PRF (Alleroed, Denmark). However, as the name implies it is not a PRF but produces a PRP product.[12]

2000

Choukroun et al introduced PRF and categorized as a "second-generation" platelet concentrate. This proved an important milestone in the evolution of terminology.[13]

2006

Bielecki et al and Cieslik-Bielecka et al proposed to define PRP as inactive substance, while PRG (Platelet Rich Gel) was a more biologically activated fibrin matrix rich in platelets, leukocytes and relative active molecule. Sacco 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. The fibrin rich blocks that were obtained were much larger, richer and denser.[14,15,16,17]

2008

Everts et al 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 labeled platelet-leukocyte-gel" (PLG).[18,19]

2009

Dohan Ehrenfest et al. proposed first classification about platelet concentrate[12] using 2 key parameters: Leukocytes and the fibrin architecture: (1) Pure platelet-rich plasma (P-PRP) - or leukocyte-poor platelet rich plasma (LP-PRP); (2) Leukocyte-and platelet-rich plasma (L-PRP); (3) Pure PRF (P-PRF) - or leukocyte-poor PRF; and (4) Leukocyte- and platelet-rich fibrin (L-PRF).

2010

Concept of sticky bone (autologous fibrin glue mixed with bone graft) was given by Sohn in 2010.[20]

2012

Mishra et al. proposed a classification only for sports medicine applications and taking into consideration the platelets and leukocytes concentrations. This classification creates 4 types of PRP, depending on the presence or absence of leukocytes and on the activation or not of the PRP.

Following this proposal-

- Type 1 PRP is a L-PRP solution
- Type 2 PRP is a L-PRP gel
- Type 3 PRP is PPRP solution
- Type 4 PRP is a P-PRP gel

This classification follows therefore the same idea than the general classification published in 2009, but is more limited (PRP only) and less intuitive (types of PRPs are less obvious than clear terminology).

The only new parameter of this classification is the evaluation of the platelet concentration; type A PRP being 5-time or more the blood concentration of platelets, and type B PRP being less than 5 times the blood concentration of platelets. This last parameter is debatable, as the concept of platelet concentration was largely abandoned in the previous years for a logical reason: platelet concentration depends only on the volume of liquid serum used to keep the platelets in suspension. The quantity of serum varies a lot depending on the protocol and the expected application, and has no impact on the expected effect. The concept of absolute quantity of platelets would be more logical, even if most publications failed to detect a clear and reproducible impact of this parameter in the clinical outcomes. From this standpoint the 5-time threshold has no consensual sense and justification.

Another system called PAW (Platelets, Activation, White cells) was proposed in 2012 to organize and compare results in the literature, and it insists on the platelet quantity (absolute number), the activation mode of the platelets and the presence of white cells. This system again is limited and only covers the PRP families, and is in fact very similar to the proposal of ***Mishra et al***. Leukocytes and activation (liquid or gel) are already well isolated parameters, and the question of the platelet quantity remains still a significant debate, as no publication was really able to define what would be an optimal platelet quantity, or even if the concept really exists with complex multi-components materials such as platelet concentrates.[22]

2014

Choukroun[23] invented an advanced PRF (APRF). Tunalı et al[24] introduced a new product called T-PRF (Titanium prepared PRF).

2015

Mourão et al introduced i-PRF.[25]gave detailed technical note on preparation of i-PRF

Method of preparation of L-PRF

Choukroun's PRF protocol is simple technique developed in France by Choukroun J., Adda F., Schoeffler C., Vervelle A.(2001). Venous blood is collected in dry glass tubes and centrifuged at 3000 rpm (Process protocol, Nice, France). In the absence of anticoagulants, 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. The PRF clot forms a strong fibrin matrix with complex three dimensional complex architecture, with most of the platelets and leucocytes from the harvested blood are concentrated.[26] The PRF clot is then placed on the grid in the PRF Box (Process Ltd., Nice, France), and covered with the compressor and lid. This produces an inexpensive autologous fibrin membrane in approximately 1 min. The PRF Box produces membranes of constant thickness (1mm) that remain hydrated for several hours and recovers the serum exudate expressed from the fibrin clots which is rich in the proteins vitronectin and fibronectin. The exudate collected at the bottom of the box may be used to hydrate graft materials, rinse surgical sites, and store autologous grafts.

However, another alternative to obtain a PRF membrane is by pressing the clot between two gauzes thereby squeezing out the fluids in the fibrin clot (Raja and Naidu 2008).

The PRF clot can also be placed into the cylinder in the PRF Box and slowly compressed with the piston which results in "plugs" or thick small discs of PRF measuring 1 cm in diameter. These are useful in protecting extraction sites.

Method of preparation of i-PRF –

To obtain the i-PRF, blood collection was performed using 9 ml tubes without any additive (Dry Vacutube, Biocon®, Brazil). After collecting three tubes, they were placed in the horizontal centrifuge (B-40, RDE®, Brazil), with a tube filled with water in order to maintain the balance during centrifuging for two minutes at 3300 rpm. Upon termination of this process, it is possible to observe an orange color area in the tube (i-PRF) and the remaining blood materials below. Then, the tubes were opened carefully, to avoid homogenization of the material. We collected 5 ml of i-PRF from the tubes using a 20ml syringe (Injex®, Brazil) with a 18G hypodermic needle (Injex®, Brazil).[27]

Method of preparation of T-PRF-

Intravenous blood was collected in a 10 ml sterile titanium test tube without anticoagulant by venipuncturing of antecubital vein. The tubes were 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 acellular plasma (Platelet Poor Plasma (PPP) at the top. T-PRF clot thus formed was separated using sterile tweezers and scissors and transferred onto PRF box and a stable fibrin membrane was obtained by squeezing serum out of the T-PRF clot.[28]

PRF application-

- In Oral and Maxillofacial Surgery
- In Periodontics
- In Endodontics
- In Plastic Surgery
- In Medicine

In Oral and Maxillofacial Surgery-

- Sinus floor augmentation
- Ridge augmentation
- Protecting extraction site
- Management of trismus
- Implant site preparation
- Lateral window osteotomy procedure

In Periodontics –

- Treatment of gingival recession
- As a palatal bandage
- Reconstruction of lost interdental papilla
- Treatment of intrabony defects
- As a sole grafting material
- Treatment of furcation defects
- Treatment of electrosurgery induced osteonecrosis
- Treatment of osseous defects in aggressive periodontitis
- Complement healing of donor site
- Treatment of peri-implant defects

In Endodontics –

- Treatment of periapical lesion
- Revitalization of tooth with necrotic pulp and open apex

In Plastic Surgery –

- Facial esthetic lipostructure
- Periorbital treatment
- Treatment of tear toughs and supraorbital hollow
- Glabellar furrows
- Mid face and lower face treatment (malar augmentation)
- Zygomatic arch enhancement
- Correction of nasolabial fold and marionette folds
- Treatment of rolling acne scars and oxcar acne scars

In Medicine-

- For regeneration of chronic leg ulcers
- For cartilage (knee) regeneration
- For the regeneration of ligaments and tendons
- Orthopedic medicine

Merits of PRF-

1. Easy to prepare and use[26]
2. No biochemical modification[26]
3. Cost effective process[29]
4. Long term effect[26]
5. Support cytokines enmeshment and cellular migration[26]
6. Amplified incorporation of the circulating intrinsic cytokines in the fibrin meshes[26]
7. An immune organizing node[26]
8. Due to slow polymerization supports and accelerates the healing process[26]
9. Helps in hemostasis[30]
10. Elasticity and flexibility due to three dimensional structure of PRF membrane[30]

Demerits of PRF-

1. Less amount of PRF is obtained, because of autologous blood[29]
2. The clinical advantage of PRF depends on time gap between speed of handling between blood collection and centrifugation as PRF is prepared without any addition anticoagulants[31]
3. The fibrin matrix contains all the highly antigenic plasmatic molecules and the circulating immune cells, that is why PRF is totally specific to the donor[3]
4. Should be used immediately after preparation as it will shrink resulting in dehydration altering the structural integrity of PRF and leukocyte viability will be adversely affected altering its biologic properties[32]
5. Risk of bacterial contamination when stored in refrigerator [31]

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