Review Article

Received : 15-06-16
Review completed : 19-08-16
Accepted : 07-09-16

PRF – AN INNOVATIVE PROCEDURE IN DENTISTRY

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ABSTRACT

Platelet rich fibrin (PRF) is an autogenous biomaterial consisting of growth factors and cytokines entrapped in a fibrin matrix. It combines the fibrant sealant properties along with growth factors thereby providing an ideal environment for wound healing and regeneration of tissues. The present review discusses about PRF and its uses in dentistry in a wide range of treatment modalities.

KEYWORDS: Platelet Rich Fibrin [PRF], growth factors, healing, Dentistry.

INTRODUCTION

Platelets' regenerative potential was introduced in the 70's[1], when it was observed that they contain growth factors that are responsible for increase collagen production, cell mitosis, blood vessels growth, recruitment of other cells that migrate to the site of injury, and cell differentiation induction, among others[2]. One of the latest innovations in oral surgery is the use of platelet concentrates for in vivo tissue engineering applications: 1) platelet-rich plasma [PRP] and 2) platelet-rich fibrin (PRF). Platelet concentrates are a concentrated suspension of growth factors found in platelets, which act as bioactive surgical additives that are applied locally to induce wound healing[2]. Whitman et al[3], in 1997, were the first to introduce the use of platelet-rich plasma in oral surgical procedures, reporting great advantages because it enhances osteoprogenitor cells in the host bone and bone graft. However, using it also presents risk because bovine thrombin, which is used to handle PRP, may generate antibodies to factors V, XI, and thrombin that could cause coagulopathies that may endanger life[2]. On the other hand, PRF was first used in 2001 by Choukroun et al.[4], specifically in oral and maxillofacial surgery, and is currently considered as a new generation of platelet concentrate. It consists of a matrix of autologous fibrin[5] and has several advantages over PRP, including easier preparation and not requiring chemical manipulation of the blood, which makes it strictly an autologous preparation[2].

PREPARATION OF PRF

For preparation of PRF, blood sample is collected from the patient without anticoagulant using a butterfly needle and 10 ml blood collection tubes. After collection of blood, it is immediately centrifuged on a table-top centrifuge at a rate of 3000 rpm for 10 minutes. After centrifugation, 3 layers are obtained in the test tube. The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The middle layer of PRF clot is then removed with sterile tweezers and separated from the underlying RBC layer using scissors and then transferred on a sterile dish and stored in a refrigerator. It is supposed that the junction of PRF to the RBC layer is rich in growth factors and therefore this region is preserved.[6] PRF results from a natural and progressive polymerization which occurs during centrifugation [7]. Because of the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful preparation of PRF, speedy blood collection and immediate centrifugation, before the clotting cascade is initiated, and is absolutely essential[8]. The slow handling of blood to centrifugation process will result in diffuse polymerization of fibrin leading to the formation of a small blood clot with irregular consistency[9].Also, PRF membrane can be obtained by squeezing out the liquids present in the fibrin clot. Liquid removal from the PRF fraction can be done through mechanical pressure between gauze layers resulting in a fairly solid, gel-like material that can be used in various clinical applications as a filling material or as a

suturing membrane[10]. PRF membrane can also be prepared by compressing PRF clot in special tools like "PRF Box" resulting in standardized membranes of constant thickness and size along with PRF exudate. PRF exudate contains good amount of growth factors (TGF-b1, PDGF-AB, VEGF etc.), matrix glycoproteins (fibronectin, vitronectin etc.) and proteins specialized in increasing cell attachment to biomaterials and titanium and therefore can be used for biomaterial impregnation, rinsing surgical sites, hydration of graft materials and for storage of autologous grafts[11].

ADVANTAGES

1. Its preparation is a simplified and efficient technique, with centrifugation in a single step, free and openly accessible for all clinicians.
2. It is obtained by autologous blood sample.
3. Minimized blood manipulation.
4. It does not require the addition of external thrombin because polymerization is a completely natural process, without any risk of suffering from an immunological reaction.
5. It has a natural fibrin framework with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration effectively.
6. It can be used solely or in combination with bone grafts, depending on the purpose.
7. Increases the healing rate of the grafted bone.
8. It is an economical and quick option com- pared with recombinant growth factors when used in conjunction with bone grafts.
9. Used as a membrane, it avoids a donor site surgical procedure and results in a reduction in patient discomfort during the early wound-healing period .
10. The studies of PRF present it to be more efficient and with less controversies on its final clinical results when compared to PRP.[12-16]

DISADVANTAGES

1. The final amount available is low because it is autologous blood.
2. The success of the PRF protocol depends directly on the handling, mainly, related to blood collection time and its transference for the centrifuge.
3. Need of using a glass-coated tube to achieve clot polymerization .
4. Possible refusal of treatment by the puncture required for blood collection.

Only needs a minimal experience of clinician for PRF manipulation.

CURRENT APPLICATION OF PRF IN DENTISTRY

Majority of the research has been concentrated on the use of PRF in oral surgery for bone augmentation, sinus lifts, avulsion sockets etc and in periodontics to correct intra-bony defects, gingival recession, guided bone regeneration, periapical lesions etc. It has also been used for regeneration in open apex, regenerative pulpotomies, periapical surgeries etc.

- Endodontics : Studies have shown that PRF can be used as a scaffolding material in an infected necrotic immature tooth for pulpal regeneration and tooth revitalization[17]. Also, some case reports show that the combination of PRF membrane as a matrix and MTA in apexification procedures prove to be an effective alternative for creating artificial root-end barriers and to induce faster periapical healing in cases with large periapical lesions. Use of a membrane can prevent the extrusion of material. Use of PRF in regenerative pulpotomy procedures have also been documented where coronal pulp is removed and the pulp wound is covered by PRF followed by sealing it with MTA and GIC . PRF has also been used to fill in the bony defects after periapical surgeries like root end resection etc. 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. The potential theory behind the success of the use of PRF for regeneration of open apex could be attributed to a study conducted by Huang et al, who

concluded that the PRF causes proliferation of human Dental Pulp Cells and increases the protein expression of these Dental Pulp Cells differentiate into odontoblasts like cells. OPG and ALP expression are generally regarded as markers of odontoblastic differentiation [17].

- Periodontics : In periodontics, PRF has been used to treat gingival recession, intra-bony defects and periapical lesions. Some case reports show the use of a combination of PRF gel, hydroxyapatite graft and guided tissue regeneration (GTR) membrane to treat IBD . Some studies show the use of PRF gel and PRF membrane in combination with a bone graft for treating a tooth with a combined periodontic- endodontic lesion[18]. Some studies show use of two layers of PRF membrane with to cover the defect. The membranes are very thin and inhomogeneous and leucocytes and platelet aggregates are believed to be concentrated in end of the membrane. Therefore, two layers of membrane in opposite sense can be used to prevent the resorption of the thin membrane and to allow the entire surgical area to be exposed to same components (leucocytes and platelet aggregates). Platelet rich fibrin as a potential novel root coverage approach has been reported by Anil kumar et al. for covering localised gingival recession in mandibular anterior teeth using combined laterally positioned flap technique and PRF membrane.
  PRF can promote the healing of osseous defects by the following mechanisms. According to Chang et al. PRF promotes the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegerin (OPG) which in turn causes proliferation of osteoblasts. Another study by Huang et al. reported that PRF stimulates the osteogenic differentiation of the human dental pulp cells by up regulating osteoprotegerin and alkaline phosphatise expression. PRF also releases growth factors such as platelet-derived growth factor and transforming growth factor which promote periodontal regeneration.

- Oral and Maxillofacial Surgery: Studies show that PRF can be used as filling material in extraction sockets. As a filling material in extraction sockets, PRF will act as a stable blood clot for neovascularization and accelerated tissue regeneration. This can be used to improve wound healing in immunocompromised and diabetic patients. Also, as PRF stimulates coagulation (with thrombospondin) and wound closure, it can be used as an adjuvant in patients on anticoagulant therapy [11] PRF has been extensively used in sinus lift procedures. Some studies show the use of PRF as the sole filling material during sinus lift and implantation. Some studies show the use of PRF in combination with other bone graft materials in various direct and indirect sinus lift techniques like bone-added sinus floor elevation, osteotome-mediated sinus floor elevation, minimally invasive antral membrane ballon elevation etc . Some studies also show the use of PRF in combination with beta Tricalcium phosphate (beta TCP) without bone graft in sinus lift procedures and chronic periodontal lesions. The filling of avulsion sockets with PRF leads to very favourable results when bony walls are intact. A combination of PRF with bone substitutes and other adjuncts may be necessary in residual defects where one or several walls are missing or damaged in order to provide an adequate reconstruction of bone volume. PRF increases the cohesion between the graft materials as fibrin act as physiological glue between the wound tissues. Natural blood coagulation leads to formation of a fibrin matrix that biologically links wounded tissue together along with cell proliferation, cell migration, neomatrix apposition and remodelling. Therefore, the combination of PRF with other graft materials should improve the integration of graft material, since PRF is an optimized blood clot.In cases of wide sockets and lesions where primary closure is difficult, PRF membrane can be used as a covering and protective

membrane that promotes re-epithelialization of the site and accelerates the merging of the wound margins. The elasticity and strength of PRF fibrin membrane makes it easy to suture. As a membrane for guided bone regeneration (GBR), the PRF dense matrix architecture covers, protects and stabilizes bone graft material and operative site in general.

- Tissue engineering: The use of PRF as a tissue engineering scaffold was investigated by many researchers for the past few years. In a study by Gassling et al. reported that PRF appears to be superior to collagen as a scaffold for human periosteal cell proliferation and PRF membranes can be used for in vitro cultivation of periosteal cells for bone tissue engineering. Thus PRF is a potential tool in tissue engineering but clinical aspects of PRF in this field requires further investigation.

ROLE OF PRF IN WOUND HEALING

PRF consists of a fibrin matrix polymerized in a tetra molecular structure with the incorporation of platelets leukocyte and cytokines, and the presence of circulating stem cells. PRF stimulates osteoblasts, gingival fibroblasts, and periodontal ligament cells proliferation as a mitogen. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts . It permits a rapid angiogenesis and an easier remodelling of fibrin. PRF matrix can release various growth factors and cytokines locally at the wound site for a prolonged period of time which play important role in various stages of wound healing promoting periapical tissue generation. Growth factors are released from the alpha-granules in the platelets when they are activated, secreted, or aggregated by collagen or epinephrine. Platelet-derived growth factor (PDGF) is a potent activator for cells of mesenchymal origin. It is among the first cells to reach at the wound site. Strayhorn et al suggested that PDGF might act mostly on osteoblastic cell proliferation, exerting most of its effects during the early phases of wound healing It also stimulates chemotaxis, proliferation, and new gene expression in monocytes-macrophages and fibroblasts in vitro, cell types considered essential for tissue repair.Vascular endotheilial growth factor (VEGF) is a major angiogenic growth factor. It acts on endothelial cells, being produced by numerous cell types, including vascular smooth muscle cells (VSMC), fibroblasts etc. initiating blood vessel formation. Transforming growth factor Beta-1 (TGF beta-1), an inflammatory regulator, is the most powerful fibrosis agent amongst all cytokines and can induce a massive synthesis of collagen and fibronectin either by fibroblasts or osteoblasts .[9,19]The physiologic fibrin matrix of PRF, obtained as the result of slow polymerization, has the ability to hold various growth factors and cytokines and release them at the wound site for a prolonged time period. Moreover, the fibrin matrix itself shows mechanical adhesive properties and biologic functions like fibrin glues: it maintains the flap in a high and stable position, enhances neoangiogenesis, reduces necrosis and shrinkage of the flap, and guarantees maximal root coverage. It plays an important role in angiogenesis and wound coverage. Angiogenesis requires an extracellular matrix to allow migration, proliferation and phenotype differentiation of endothelial cells. The angiogenesis property of the fibrin matrix is explained by the 3-dimensional structure of the fibrin gel and the simultaneous action of the cytokines trapped in fibrin meshes. Fibrin matrix guides the wound coverage affecting the metabolism of fibroblasts and epithelial cells. The epithelial cells around the wound margins lose their basal and apical polarity and produce basal and lateral extensions towards the wound site. These cells then migrate onto the transitory matrix made by fibrinogen, fibronectin, tenascin and vitronectin . Fibrin, fibronectin, PDGF and TGF-B are essential to modulate integrin expression, fibroblast proliferation and their migration inside the wound. After migration and degradation of

fibrin, fibroblasts start the collagen synthesis.PRF also aids in trapping circulating stem cells brought to the wound site due to initial neovascularization during hemostasis and healing. Set in the fibrin matrix, these cells converge on a secretory phenotype, allowing the vascular and tissue restoration. This aspect of PRF serving as a net to the stem cells can be beneficial in cases of wide defects.[20]

CONCLUSION

Although PRF belongs to a new generation of platelet concentrates, the biologic activity of fibrin molecule is enough in itself to account for significant cicatricial capacity of the PRF. The slow polymerization mode confers to PRF membrane as a particularly favorable physiologic architecture to support the healing process. However, it is now necessary to look further into platelet and inflammatory features of this biomaterial. Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol.

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