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PLATELET RICH FIBRIN IN DENTISTRY- A REVIEW

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Abstract: -

Platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane. In the present review article, the evolution, preparation of PRF has been described. Autologous PRF is considered to be a healing biomaterial, which has its numerous applications in various disciplines of dentistry.

Keywords: - *Platelet rich fibrin, Growth factors.*

INTRODUCTION

Regeneration of lost periodontal tissue has become the primary therapeutic goal in periodontics. The objectives of periodontal regenerative therapy is to regenerate new bone, cementum, and periodontal ligament on a previously diseased root surface. Numerous therapeutic modalities for regeneration of periodontal defects have been investigated[1]. Periodontal regeneration is a multifactorial process and requires an orchestrated sequence of biological events including cell adhesion, migration, proliferation, and differentiation.[2] Significant progress in the understanding of periodontal wound healing has been made. Although complete regeneration of the periodontal attachment apparatus, including bone, periodontal ligament, and cementum is possible, it is still not predictable.[3] Currently, there are variety of treatment modalities available for periodontal regenerative therapy, which include autografts,[4-8] demineralized freeze-dried bone allografts (DFDBAs),[9] bovine-derived xenografts,[10] barrier membranes,[11-14] and combinations of membranes and bone grafts.[15-16] Although these regenerative materials are still used today, the introduction of biomimetic agents, such as enamel matrix derivatives,[17] platelet rich plasma (PRP),[18] platelet-derived growth factor,[19-21] and bone morphogenetic proteins,[17] have shown better outcomes for periodontal regeneration.

Recently, polypeptide growth factors which are biological mediators that have the ability to regulate cell proliferation, chemotaxis and differentiation have been introduced. Several polypeptide growth factors have been identified in human periodontal tissues by immunohistochemistry and in situ hybridization.[22] Therefore, polypeptide growth factors have a potential application in periodontal wound healing by promoting regeneration of periodontal tissues. Polypeptide growth factors have been shown to promote cell growth and differentiation in vitro and to induce periodontal regeneration in animals.[23-32] Human periodontal regeneration studies examining the effectiveness of polypeptide growth factors, used alone or in combination with other materials and techniques, have been conducted with autologous platelet-rich plasma and recombinant platelet-derived growth factor and reported favorable results.

Autologous blood concentrates constitute a safe and convenient approach to deliver high concentrations of polypeptide growth factors to periodontal surgical wounds. Among platelet concentrates, platelet-rich fibrin (PRF) belongs to a group of second-generation blood autologous preparations that was originally described by Choukroun et al (2001).[33] Platelet-rich fibrin is obtained by gentle centrifugation of peripheral blood and is characterized as being leukocyte and platelet rich and fibrin dense,[34-36] besides not requiring the addition of any anticlotting agent. Dohan Ehrenfest et al (2010)[37] showed that approximately 97% of platelets and 50% of leukocytes of the original blood volume were concentrated and three dimensionally distributed in the PRF clot, which is one of the three layers resulting from the centrifugation process. After its preparation and collection, PRF can be used directly as a filler agent or compressed into a membrane. In either of those applications, PRF is believed to release polypeptide growth factors, such as transforming growth factor-b1, platelet-derived growth factor, vascular endothelial growth factor and matrix glycoproteins such as thrombospondin, into the surgical wound in a sustained fashion for at least 7 days in vitro.[38]

PRF looks like a fibrin network and leads to more efficient cell migration and proliferation, and thus cicatrization.[39] This unique structure may act as a vehicle for carrying cells that are essential for tissue regeneration. A latest, study has demonstrated that the PRF membrane has a very significant slow sustained release of key growth factors for at least one week and up to 28 days,[40] which means that the membrane stimulates its environment for a significant time during wound healing. In addition, PRF has been shown to act as suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering applications.[41]

The application of PRF in fields of plastic surgery, [42] oral and maxillofacial surgery [43] and implant surgery [44] has demonstrated successful and rapid results in terms of bone regeneration. The field of periodontal therapy has just started to explore the vast benefits of PRF for management of various types of periodontal defects. Recently various investigators have examined efficacy of PRF for the regeneration of various types of periodontal defects [45-47] including alveolar ridge preservation [48-50] and sinus augmentation.[51-52] Therefore, attempt has been made to present update literature on present status of PRF in regeneration of various types of periodontal defect and effectiveness of PRF for regeneration of bone during adjunctive implant therapy. In addition, the current evidence for the role of PRF in tissue engineering has been added.

Evolution of Various Platelet Concentrates

Platelets are anucleate cytoplasmic fragments containing α-granules that are spherical or oval structures with diameters ranging from 200 to 500nm.[59] They form an intracellular storage pool of proteins vital to wound healing, including platelet-derived growth factor (PDGF), transforming growth factor (TGF β), and insulin-like growth factor (IGF-I). Secretion of the active proteins follows the fusion of the granules with the platelet cell membrane, which subsequently bind to the transmembrane receptors of the target cells.[59] Numerous techniques of autologous platelet concentrates have been developed and applied in oral and maxillofacial surgery. The first generation incorporates the platelet-rich plasma while the second generation involves the platelet-rich fibrin. Platelet-rich plasma was introduced for the first time by Marx et al in 1998.[60] PRP was used in mandibular reconstructions along with cancellous bone marrow grafts. The data reported by Marx suggested that PRP addition accelerated the rate and degree of bone formation. PRP was developed to combine the fibrins sealant properties with growth factor effects of platelets, thus providing an ideal growth factor delivery system at the site of injury.

These growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, regeneration, and cell proliferation.[61] However, lack of uniformity in PRP preparation protocol as different platelet concentrations have different storage time, release of growth factors for a shorter period of time and antibodies to bovine factor Va may cross react with human factor Va and may produce coagulopathies and rare bleeding episodes.[62]

PRF

Concept and Evolution of PRF: A second-generation platelet derivative developed in France by Choukroun et al because, unlike other platelet concentrates like PRP, this technique does not require anticoagulants nor bovine thrombin or any other gelifying agent. PRF is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines.[63] PRF represents a novel measure in the therapeutic concept with elementary processing and absence of artificial biochemical modification like the use of bovine thrombin.[64] The crux of PRF synthesis lies in the attempt to accumulate platelets and release cytokines in a fibrin clot. The PRF clot is yielded by a natural polymerization process during centrifugation, and its natural fibrin architecture seems responsible for a slow release of growth factors and matrix glycoproteins during ≥7 days.[65] Such a slow release is unimaginable to point out in most PRP techniques because of their brutal platelet activation, continous release of growth factors, and very light fibrin network produced to sustain the concentrate injection.[66] The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest[67-68] and shows a complex architecture as a healing matrix with unique mechanical properties which makes it distinct from other platelet concentrates. PRF enhances wound healing and regeneration and several studies show rapid and accelerated wound healing with the use of PRF than without it.[69-70] PRF is superior to other platelet concentrates like PRP due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous compounds like bovine thrombin and calcium chloride. It is advantageous than autogenous graft also because an autograft requires a second surgical site and procedure. Thus, PRF has emerged as one of the promising regenerative materials in the field of periodontics.

Biological Features

PRF dwells among a new generation of platelet concentrate that jump starts the healing process to maximize predictability. It consists of the platelets, cytokines, and the fibrin matrix. Platelets and leukocyte cytokines play an important part in the biology of this biomaterial.[71] Degranulation of platelets entails the release of cytokines, able to stimulate cell migration and proliferation within the fibrin matrix, launching the first stages of healing.[72-73] Fibrin matrix supporting them constitutes the determining element responsible for the real therapeutic potential of PRF. The biologic activity of the fibrin molecule highlights its significant cicatricial capacity. However, a detailed understanding of the constituents of PRF and their biological role will help understand this biomaterial from a clinical standpoint and subsequently extend the fields of therapeutic applications.

Functions of platelet rich fibrin (PRF)

Fibrin is an activated form of fibrinogen molecule present in plasma as well as the α-granules of platelets. It plays a significant role in platelet aggregation and achievement of hemostasis. The soluble fibrinogen is transformed into insoluble fibrin that polymerizes to a cicatricial matrix.[68] The slow and natural polymerization of fibrin results in its homogenous 3-dimensional organization during the centrifugation performed in PRF preparation. This leads to the intrinsic incorporation of platelet cytokines and glycan chains in the fibrin meshes. The fibrin matrix present in PRF is flexible, elastic, and very strong.[75] It consists of weak thrombin concentrations which entails equilateral junctions. These connected junctions permit the ecesis of a fine and flexible fibrin network capable of supporting cytokines and cellular migration that occurs. This results in an increase in the life span of these cytokines as their release and use will occur at the time of initial cicatricial matrix remodeling. Thus, the cytokines are made available for a mandatory period required by the cells to initiate the healing. Fibrin meshwork in PRF differs from that in PRP. In PRP, there are bilateral junctions resulting in a rigid network that does not honor the cytokine enmeshment and cellular migration. The increased thrombin required for rapid setting of the PRP leads to a rigid polymerized material.[75]

Preparation of PRF-

Preparation of PRF follows the protocol developed by Choukroun et al. in Nice, France.[76] The protocol for PRF preparation is very simple; however, it has to be manufactured just prior to its application.

The materials required for preparation of PRF are:
Table centrifuge, 10-mL dry glass test tube (without anticoagulant), Blood collection armamentarium.
The main advantages in PRF preparation are the single stage centrifugation and absence of bovine thrombin. The blood obtained from the subject is placed into the test tube and centrifuged immediately for 10 minutes at 3000 rpm.[68] Others have used 2700 rpm for 12 minutes [78] with similar findings. The steps involved are as follows:

Blood specimen is collected or drawn from the patient, The blood specimen is placed in the centrifuge and is allowed to spin immediately for the stipulated time, following this the blood sample settles into various layers.
The absence of any anticoagulant grants the activation of platelets to set off a coagulation cascade. Due to the absence of the anticoagulant, the blood coagulates immediately upon contact with the glass tube. Initially, fibrinogen occupies the upper part of the tube, only till the circulating thrombin transforms it into a fibrin network.[79]
The layers that are formed are as follows: The lower fraction containing the RBCs, the middle fraction containing the fibrin clot, The upper fraction containing the straw-colored acellular plasma.
The upper portion of the test tube containing the acellular plasma is removed. The middle portion containing the fibrin clot is then removed and is scrapped off from the lower part containing the red blood cells. The natural and progressive polymerization results in a fibrin clot formation with substantial embedding of platelets and leukocyte growth factors into the fibrin matrix.[80]

Preparation of PRF Membrane:
The clot can be firmly pressed between two-gauge pieces to obtain an inexpensive autologous fibrin membrane. The serum exudate expressed from the clot is rich in proteins such as vitronectin and fibronectin.[67] This exudate may be used to hydrate graft materials, rinse the surgical site, and store autologous graft. The PRF Box (Process Ltd., Nice, France) is available to prepare the PRF membrane. The PRF clot is placed on the grid in the PRF box and covered with compressor lid which squeezes out the fluid from the clot. The membranes formed using this method had constant thickness which remain hydrated for several hours and have recovered the serum exudate expressed from the fibrin clots.

Growth factors present in PRF[82]:
2. The functions of Platelet-derived growth factor (PDGF) Migration and proliferation of
1. Function of Transforming growth factor-β (TGF-β) Released from α-granules of platelets,Stimulates proliferation of osteoblasts, Synthesis of collagen type I and fibronectin ,Enhanced woven bone formation, Enhanced chemotaxis of osteoblast cells. mesenchymal lineage cells, angiogenic effect on endothelial cells.
3. Vascular endothelial growth factor (VEGF) initiates angeogenesis

Clinical applications-
4.Insulin growth factor-1 (IGF-1) Stimulates osteoblast proliferation, Chemotactic effects towards human osteoblasts, Increased expression of osteocalcin, enhances wound healing.
5.Fibroblast growth factor Stimulates osteoblast proliferation, Chemotactic effects towards human osteoblasts, increases expression of osteocalcin, enhances wound healing

1. Oral surgery:
- As filling material in extraction sockets. o To improve wound healing in immunocompromised and diabetic patients.
- Stimulates coagulation (with thrombospondin) and wound closure, it can be used as an adjuvant in patients on anticoagulant therapy.
- Sole filling material during sinus lift and implantation o 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.[83]
- Increases the cohesion between the graft materials as fibrin act as physiological glue between the wound tissues.
- 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

2. Periodontics:
- To treat gingival recession, intra-bony defects and periapical lesions.
- Use of a combination of PRF gel, hydroxyapatite graft and guided tissue regeneration (GTR) membrane to treat Intra Bony Defect o PRF gel and PRF membrane in combination with a bone graft for treating a tooth with a combined periodonticendodontic lesion
- Promote the healing of osseous defects[84]

3. Endodontics:
- PRF can be used as a scaffolding material in an infected necrotic immature tooth for pulpal regeneration and tooth revitalization.
- It can be used with the combination of PRF membrane as a matrix and MTA in apexification procedures prove to be an effective different for creating artificial root-end barriers and to induce faster periapical healing in cases with large periapical lesions.
- PRF is used to fill in the bony defects after periapical surgeries like root end resection.[84]

4. Tissue engineering:
o 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.[84]

Conclusion-
- Regeneration is a viable treatment modality that allows continued root development of immature teeth with open apices and necrotic pulps. Platelet-rich plasma appears to be a suitable scaffold for regeneration of vital tissues in teeth with a necrotic pulp and an associated periapical lesion. Regenerative endodontic procedures may offer an effective treatment option to save teeth with compromised structural integrity.

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