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Platelet-Rich Fibrin – A Review of Its Impact in Endodontics

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Abstract

As with every aspect of science, Endodontics is in a perpetual quest for the best outcome. In Endodontic surgery, this entails a faster and predictable healing, of both soft tissue and hard tissue. One of the recently reported adjuncts that has the potential to achieve this objective is platelet-rich fibrin (PRF). PRF is one among a group of compounds derived from blood platelets, with a positive influence on wound healing, by virtue of their content of growth factors. This paper is aimed at a detailed description of the preparation, effects and endodontic applications of PRF.

Key words: *Platelet derivatives; Platelet-rich fibrin; Endodontics; Surgery; Regeneration*

Introduction

Endodontics is a science evolving at a fast rate, primarily because of its prudence to imbibe cutting edge technology from fields as varied as material science, biomechanics, or molecular biology. The conventional dimensions of endodontic surgery are now challenged by more precise and predictable microsurgical techniques [1]. Advances in tissue regeneration have also fuelled the research in total pulp regeneration. A zone of convergence for both these realms of Endodontics is constituted by plasma-derived materials. Platelet-rich plasma (PRP) had been found to be an effective scaffold for surgical and regenerative endodontic procedures, although the elaborate process of preparation of PRP involving chemicals and bovine thrombin became a major reason for the decline in its popularity. In its place, platelet-rich fibrin (PRF) is now being employed by researchers world-wide in many

clinical situations. This article details the rationale for the use of PRF in endodontics and explores the future prospects for this material. Articles on PRF published within the last decade were searched in literature databases using relevant keywords. A few articles were also included following a manual search. The extracted information has been summarized and structured as follows.

Platelet Concentrates– A Historical Perspective

The first group of platelet-derived materials to be described in literature almost 40 years ago was designated as fibrin glues. They composed primarily of concentrated fibrinogen, the polymerisation of which was induced by thrombin and calcium [2]. They were hailed as a revolutionary replacement to the conventional haemostatic agents such as collagen sponges, oxidized cellulose or synthetic adhesives [3]. Fibrin adhesives involved the natural biologic coagulation mechanisms, amplified artificially [3]. They constituted a good choice in surgeries, especially when derived from autologous sources. However, being blood-derived, they carried an inherent risk of viral contamination [3]. Another disadvantage was the low concentration of fibrinogen, thus lowering the stability and quality of the glues [4]. Moreover, the complexity and cost of the production protocols discouraged their universal application [3]. These glues have since been replaced by platelet concentrates.

The standard platelet concentrate used mainly for transfusions typically contains $0.5x10^{11}$ platelets and has been referred to as Platelet-rich-Plasma or PRP [2]. Several preparations of platelet concentrates synthesised by similar techniques have been described in literature [3,4,5]. All these techniques involve collection of blood from the patient, addition of anticoagulant and centrifugation to separate the red blood corpuscles (RBC), plasma and buffy coat containing platelets. The steps that follow vary according to the protocol employed and are aimed at segregating the platelet concentrates from the other components. Each technique yields a different product with different biology and potential uses.

In an exhaustive review, Dohan et al have described and classified platelet-derived materials and concentrates on the basis of several criteria, such as technique characteristics, content of the concentrate and the nature of the fibrin network [2]. According to this classification, there are four groups of materials i.e. Pure Platelet-rich-plasma, Leucocyte & Platelet-rich-plasma, Pure Platelet-rich-fibrin and Leucocyte & Platelet-rich-fibrin. The last group, namely, Leucocyte & Platelet-rich-fibrin was first described by Choukroun*et al* and is also referred to as Choukroun's PRF [2]. It represents the simplest and most popular platelet derivative in current times, and hence, the following discussion focuses largely on Choukroun's PRF.

What is PRF?

Platelet-rich fibrin is a second-generation platelet derivative consisting of membranes of fibrin enriched with platelets and growth factors. This material has gained much popularity in Oral, ENT and plastic surgeries. It differs markedly from the other preparations by virtue of the differences in its preparation protocol.

The initial step in the preparation of any platelet concentrate is termed soft-spin and involves centrifugation of the collected blood to segregate the constituents into three distinct layers. This process is facilitated by the addition of an anticoagulant such as 3.8% sodium citrate [4]. Other reports have suggested that these agents may themselves have adverse and undesirable effects, and have recommended acid citrate dextrose (ACD) and citrate-theophylline-adenosine-dipyridamole (CTAD) as superior agents [5]. The supernatant acellular plasma forms 40% of the total volume of the centrifuged sample, while 55% is comprised of erythrocytes that settle at the bottom of the tube. A buffy coat with the platelet concentrate is sandwiched between these layers and forms the remainder volume of 5%. From these the supernatant platelet-poor plasma, buffy coat and some superficial RBCs are aspirated using a syringe. This is then subjected to a second round of centrifugation called hard-spin. The result is a concentrate of PRP and few RBCs underneath the platelet-poor-plasma. A large amount of the PPP is aspirated away leaving enough serum to maintain the PRP concentrate in suspension. To this rosy-hued suspension, thrombin (usually bovine), calcium chloride or other factors are added to stimulate platelet activation and fibrin polymerisation, and the resultant gel is applied to the indicated site [2,4]. The entire process is to be restricted to a maximum of one hour.

For the preparation of Choukroun's PRF, the first major deviation from the conventional protocol is that it does not require an anticoagulant prior to the soft-spin. The collected venous blood is taken in glass tubes and directly centrifuged at 3000 rpm (400g) for 10 minutes. The leucocyte-and-platelet rich fibrin forming the buffy coat is then isolated by hard-spin and may be applied to the site without the addition of any chemical. The PRF clot forms a strong fibrin matrix with a complex 3-dimensional architecture in which most of the platelets and leucocytes are concentrated. To transform this clot into a strong membrane, the clot may simply be pressed between two gauze pads [2]. An alternative is to leave the PRF clots in a sterile metal cup for about 10 minutes allowing the slow release of contained serum and thus compaction of the clot into a more favourable consistency [6].

The platelets are concentrated in the lower part of the fibrin clot, mainly at the junction between the erythrocytes forming the red thrombus and the PRF clot itself. Hence, PRF red extremity is of more clinical use and is more effective than the higher part of the clot [6].

Effects/Consequences of PRF Placement [6]

After the application of PRF on the surgical site, the platelets embedded in it get activated. This results in degranulation of the platelets and the release of the contained cytokines, which in turn stimulate cell migration and cell proliferation within the fibrin matrix and thus launch the first stages of healing. As for the platelets themselves, they aggregate on the wound site and interact with the coagulation mechanisms and contribute to haemostasis. There is also evidence of degranulation of the leucocytes in the PRF with concomitant release of specific cytokines, although this process is less understood. In

contrast, most of the cytokines released from the α granules of platelets and their effects have been extensively studied and described [7].

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Besides cytokines, platelet stimulation also releases considerable amounts of Glycosaminoglycans (GAG) such as heparin and hyaluronic acid. These are seen to be enmeshed in the PRF matrix. These glycanic links are incorporated within the fibrin polymers. They have a strong affinity for small circulating peptides such as platelet cytokines and a great capacity to support cell migrations and healing processes.

The leucocyte cytokines that have been shown to have profound effects include Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6), Interleukin 4 (IL-4), Vascular endothelial growth factor (VEGF) and Tumour Necrosis Factor alpha (TNF α). Of these IL-1 β, IL-6 and TNF α are critical in inflammatory and immune responses, while the other cytokines are associated with angiogenesis. Interestingly, VEGF is secreted by both activated leucocytes and platelets and the final increased levels of VEGF in the PRF clot may be contributed by both these cell types.

The successful extraction of PRF from a sample is determined by the speed of blood collection and transfer for centrifuge. In the absence of an anticoagulant, there would be immediate precipitation of the clotting process as the blood is collected in the glass tube. If this were allowed, the polymerization of fibrin would tend to follow a diffuse pattern, and not yield a usable clot. Such a fibrin clot would be small and with no consistency [3]. A quick centrifugation ensures the concentration of fibrinogen in the middle and upper part of the tube, providing a clinically useful clot.

Why PRF?

The rationale for the use of any platelet- or blood-derived material is based on its biologic activity. This activity comprises of tissue adherence and biodegradability. The extent of these activities has been extensively studied and may be summarized under the following heads.

Effect on dental pulp cells [7]

PRF has no cytotoxic effects on dental pulp cells and has been reported to encourage attachment of these cells to the edges of the membrane in *in vitro* conditions. It increases proliferation of these cells by acting as a mitogen. This is explained as an effect of the growth factors (TGFβ and PDGF) in PRF. Another effect that has been noted is the up-regulation of Osteoprotegerin (OPG). OPG is a natural protein that inhibits osteoclast differentiation by interfering with interaction of RANKL with RANK receptors situated on the surface of osteoclast precursors. PRF is suggested as capable of increasing the number of OPG-secreting cells and/or increasing OPG secretion by each individual cell. The effect of this up-regulation would be a decreased incidence of resorptive changes. Research has also found an up-regulation of alkaline phosphatase (ALP) mediated by PRF in dental pulp cells. This enzyme being necessary for biomineralization, its elevated levels indicates an increased differentiation of the pulp cells into odontoblasts.

Role in immune stimulation and inflammation control [8]

The key mediators of the process of immune stimulation include the leucocyte cytokines IL-1B, IL-6 and TNF α. The role of these cytokines in inflammation in the body tissues is well-established, although their quantification and description within the PRF clot is not conclusive.

Role in angiogenesis and stem cell harnessing [8]

PRF functions as a net to harness circulating stem cells attracted to the site by the beginning neovascularization. These stem cells are then stimulated to converge on a suitable phenotype such as osteoblasts, for example. This explains their potential to initiate regeneration of lost tissues. At a molecular level, the role of cytokines such as IL 4 and VEGF has been implicated in the effect of PRF on wound healing and cicatrisation. IL 4 functions by inhibiting the inflammatory signal pathways and neutralizing their amplification. This retro-control of inflammation is an interesting effect warranting much further research. On the other hand, VEGF is the most powerful vascular growth promoter and coordinates the formation of initial cicatricial structures such as vascular tubes.

The detection of pro-inflammatory and angiogenetic factors in PRF has fuelled the theory of PRF as an immune organizing node, suggesting a significant defence capacity against infections.

Antimicrobial effect [9]

A strong antimicrobial effect similar to gentamicin and oxacillin against Staphylococcus aureus (both Methicillin-sensitive and Methicillin-resistant strains) and Escherichia coli has been reported for platelet-rich derivatives. The initial explanation for this effect was based on complement receptor expression and release of oxidizing metabolites such as superoxide, hydrogen peroxide and hydroxyl radical. More recently, antimicrobial polypeptides released from platelets on thrombin-activation have been demonstrated and characterized. Also, a dose-dependent effect against Staphylococcus aureus, Escherichia coli, Candida albicans and Cryptococcus neoformans has been described by Tang et al, signifying a direct correlation between antimicrobial activity and platelet concentration. The significantly high number of leucocytes (neutrophils involved in direct bacterial killing and lymphocytes effecting antigen-specific immune response) has also been suggested to play a critical role in the antimicrobial property of PRF. Further, PRF can potentiate the antimicrobial action of antibiotics concentrated in plasma such as augmentin(amoxicillin – clavulanic acid).

Miscellaneous [9]

PRF is a recognized nidus for active substances such as serotonin, catecholamines, von Willebrand factor, proaccelerin and osteonectin. The high local concentration of these substances by the application of PRF can potentiate the already described effects as well as contribute independently to the overall healing / regenerative process.

PRF Versus other Platelet Concentrates

The methodology for preparation with critical deviations from other platelet preparations is the

primary determinant of the superior biological properties exhibited by Choukroun's PRF. The activation of conventional platelet concentrates occurs by the addition of thrombin, Calcium chloride or a suitable chemical. This releases the various biological mediators with cicatricial properties and initiates the biological activity of these concentrates. In PRF, this activation takes place spontaneously, without the addition of any chemical or foreign material. This, besides reducing concerns of probable immune reactions, also ensures a process closest to the natural sequence of events, but in an exaggerated manner. In the absence of an anticoagulant, the stimulation of platelet activity and cytokine release is massive. The polymerization of fibrin in PRF is a slow process, thus enabling intimate incorporation of platelet cytokines within the fibrin mesh. As such, these cytokines have an increased life span, i.e. they are released only at the time of initial cicatricial matrix remodelling.

The slow polymerization also allows a unique 3-dimensional structure of the formed clot. It has been described as an elastic matricial architecture exhibiting equilateral junctions between the fibrin fibrillae [3]. This in itself is favourable to cell migration and soluble molecule retention. It further aids in the critical enmeshing of the platelets and leucocytes in the mesh and is also the reason why PRF does not dissolve after application, unlike other platelet derivatives. Moreover, the resultant fibrin clot is remodelled in a pattern closely resembling that of a natural clot.

PRF is the only platelet derivative that has thick fibrin fibres due to multiple fibre assembly and thus constitutes a resistant matrix aptly described as a fibrin biomaterial.

Finally, the fibrinogen concentration in PRF is significantly higher than other preparations. This is because in addition to the fibrinogen derived from the platelet alpha granules, PRF also collects the circulating fibrinogen, thus reinforcing the final fibrin network. This high density fibrin clot can serve as a biological healing matrix by supporting cell migration and cytokine release, to a level unattainable by the other platelet derivatives.

These properties set PRF apart from the other platelet derivatives as a healing biomaterial with multiple clinical applications, rather than an adhesive alone.

Endodontic Applications

Periapical surgery

The need for a graft material in large cavitatedperiapical lesions has long been recognized. The main aim of such materials is to ensure faster and more predictable healing of the wound. This effect may be described similarly to extraction socket healing in the presence of PRF. Neovascularization and epithelial covering proceeds at a fast rate in spite of inflammatory or infective states that may prevail in the tissues. It has been reported that a cystic cavity treated with PRF exhibits complete healing in about one-sixth the time required for physiological healing [10].

PRF can be placed in these lesions either alone or in combination with other materials including Hydroxyapatite, Biodentine and osteoconductive calcium phosphate preparations. Among these, combination of platelet derivatives with

hydroxyapatite has invited much review [11]. The rationale of combining these materials is that while PRF provides the growth factors and the scaffold for tissue regeneration, the mineral grafts act as nidi for calcification and help organize the hard tissue.

Jayalekshmi et al reported bone augmentation with PRF and β-tricalcium phosphate in a case of chronic periapical cyst with 12-month follow-up. Progressive, significant and predictable clinical and radiographic bone healing without any clinical symptoms was reported. It was observed that besides promoting wound healing, bone growth, and maturation, PRF mixed with β-tricalcium phosphate bone graft had the advantages of graft stabilization, wound sealing, hemostasis, and improved handling properties [12].

In another case report, fifteen cases were presented in which conventional endodontic therapy had failed to resolve the problem and periapical root-end surgery with PRF as graft material was performed. At the end of six months, all patients had shown complete bone regeneration [13].

However, most of the reported cases have a limited follow-up period and no histological confirmation of the healing process.

Pulp regeneration

Components needed for successful regenerative endodontics include the absence of intracanal infection, a coronal seal to prevent reinfection, a physical scaffold to promote cell growth and differentiation, and signaling molecules for the growth of stem cells [14]. In a case report by Torabinejad, disinfection of the root canals was achieved with the use of 5.25% NaOCl and triple antibiotics and a PRP clot was used as the scaffold. The patient reported with symptoms of reversible pulpitis and normal periapical tissues after 14 months, whereupon, pulpectomy had to be performed. This provided an opportunity for histological evaluation of the so-called regenerated tissue. Examinations of hematoxylin-eosin–stained sections revealed the presence of a mildly cellular fibrous connective tissue, fibroblasts, and blood vessels. A few lymphocytes were observed in the specimens, and there was no evidence of odontoblasts in the sections examined. Initially, the PRP clot might have provided an excellent matrix for the placement of mineral trioxide aggregate and subsequent permanent restorations to prevent coronal leakage [15,16]. It was further theorized that since there was no step to create bleeding before placing PRP in the root canal, whatever tissue had been produced in the canal was a result of the presence of PRP [14]. The report also cited the increased concentrations of growth factors that can attract stem cells present in the apical tissues (ie, vital pulp cells, periodontal ligament, apical dental papilla, and bone marrow)and even from periapical lesions. As has been shown by Lovelace et al, the periapical tissue contains a higher concentration of stem cells compared with the blood from the systemic circulation [17]. It was thought to be likely that PRP facilitated the migration of the stem cells from the periapical tissues. Since this presents the effects of PRP in pulp regeneration, it is safe to presume that similar or perhaps better results may be achieved with PRF. However, histologic evidence to the same is presently lacking.

Endodontic-Periodontic lesions

Although traditional nonsurgical periodontal therapy and regular endodontic therapy can be predictably used to arrest mild to moderate defects, it might be inadequate for the treatment of disease characterized by deep pockets or wide circumferential apical defects caused by endodontic infection or surgery [18]. However, the major disadvantage to any procedure is that the probable outcome is tissue repair and not regeneration. The use of PRF is justified in its enhanced ability to regenerate the lost tissue/bone in all types of defects [19]. A study comparing autologous platelet concentrate with a bioabsorbable membrane in infrabony defects found similar results between the 2 groups, suggesting that it could be used in lieu of a membrane for periodontal GTR applications [18].

Limitations of PRF

The most critical limitation for the systematic use of PRF is the quantity in which it can be synthesized, as it is derived autologously immediately prior to the procedure. Long-term storage of PRF such as banking has not yet been made feasible. Further, PRF contains all circulating immune cells in the blood from which it is derived and also the antigenic plasmatic components, making it strictly donor specific. Thus PRF can never constitute an allogenic graft material [10].

Conclusion

A major setback for clinical research on PRF is the difficulty and at times improbability of obtaining histologic evidence for its success. However, clinical and radiographic evaluations indicate a favourable tissue response to this material. When considering its versatility, ease of preparation and excellent biocompatibility, PRF may be regarded as a very valuable adjunct to endodontic procedures.

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