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EVALUATION OF CLINICAL AND RADIOLOGICAL EFFECTIVENESS OF PLASMA RICH IN GROWTH FACTORS (PRGF) IN MANAGEMENT OF PERIODONTAL INTRA-OSSEOUS DEFECTS – A DOUBLE BLIND, SPLIT-MOUTH RANDOMIZED CONTROLLED TRIAL.

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ABBREVIATIONS

CAL	Clinical attachment level
FMPI	Full mouth plaque index
GML	Gingival marginal level
IBD	Intrabony defect
IBDA	Intrabony defect area
IBDD	Intrabony defect depth
IBDAF	Intrabony defect area fill
IMP	Intramarrow penetration
OFD	Open flap debridement
PDGF	Platelet derived growth factor
PPD	Probing pocket depth
PRF	Platelet rich fibrin
PRGF	Plasma rich in growth factors
PRP	Platelet rich plasma
RAL	Relative attachment level
SSBOP	Site specific bleeding on probing
SSGI	Site specific gingival index
SSPI	Site specific plaque index
TGF	Transforming growth factor
TNF	Tissue necrosis factor

ABSTRACT

Background: Plasma Rich in Growth Factors (PRGF) is a mixture of autologous proteins and growth factors, prepared from a certain volume of platelet-rich plasma obtained from a small volume of blood, which does not contain leukocytes.

Aim: The aim of the study was to evaluate the clinical and radiological efficacy of Plasma rich in growth factors (PRGF) in adjunct to open flap debridement (OFD) and intra-marrow penetration (IMP) compared to OFD and IMP alone for management of periodontal intra-osseous defects in periodontitis patients.

Material and methods: Twenty patients with forty contra-lateral sites presenting with >5mm pocket depth and >3mm of intra-bony defect component were recruited in this double blind, split-mouth randomized controlled trial. The experimental site was surgically treated with PRGF in adjunct to OFD and IMP; and the control sites were treated with OFD and IMP alone. The clinical parameters like site specific plaque index (SSPI), site specific gingival index (SSGI), probing pocket depth (PPD), relative attachment level (RAL), gingival marginal level (GML), site-specific bleeding on probing (SSBOP) were recorded at baseline, 3, 6 and 9 months. The radiological parameters like intra-bony defect depth (IBDD), intra-bony defect area (IBDA) and percentage intra-bony defect area fill (%IBDAF) was recorded at baseline, 6 and 9 months. The patient reported outcomes on swelling, bleeding and level of pain in the area treated were also assessed at day 1 to day7.

Results: No significant difference was observed for PI, GI and PPD. A Significant favorable improvement in GML was observed in PRGF treated group at all time points, suggesting continuous vertical creeping of the free gingival margin in the PRGF group at 3, 6 and 9 months. The clinical attachment gain (CAG) was significantly higher in the PRGF group at 3 months ($p=0.005$) and borderline significance at 6 months ($p=0.067$). The linear radiographic bone gain, i.e, change in IBDD, was significantly higher in the PRGF treated group at 6 months ($p=0.02$). At 6 months, the PRGF was significantly superior to the OFD (50% Vs 15%) in the number of sites that achieved CAG by 1.5 mm and linear radiographic bone gain of 1.0 mm. At 9 months, the PRGF was border-line

significance than the OFD (83% Vs 50%) in the number of sites that achieved CAG by 1.5 mm and linear radiographic bone gain of 1.0 mm.

Conclusion: PRGF was found to beneficial in terms of improved clinical attachment gain and radiographic linear bone gain, when used in adjunct to OFD and IMP for management of periodontal intra-osseous defects.

Keywords: Plasma rich in growth factors, open flap debridement, intra-marrow penetration, periodontal regeneration, intra-osseous defects, clinical trial

CHAPTER 1. INTRODUCTION

Periodontitis is a disease of the periodontium characterized by the irreversible loss of connective tissue attachment and supporting alveolar bone (1). Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host immune-inflammatory response and subsequent alterations in bone and connective tissue homeostasis (2,3). The progression of periodontitis starts from development of pocket formation induced by bacterial plaque, subsequently initiation of alveolar bone destruction, resulting in various bone destructive patterns and alteration of available alveolar bone (4). Various bone destructive patterns chiefly encountered with are horizontal defects, angular or intra-bony or intra-osseous defects, furcation defects, fenestrations, dehiscence etc.

Intra-osseous defects associated with periodontal pockets represent the anatomic squeal of the apical spread of the dental plaque in the course of periodontitis (5). Such defects are the risk factors for periodontitis progression and further loss of attachment, if left untreated (6). Because intra-osseous defects are common in periodontitis (7), there is a considerable interest in approaches that will convert such defects, at risk for disease progression, to easily maintainable shallow probing sites (8). This can be achieved by either resective (9,10) or regenerative approaches (11–13), the later considered the ideal treatment.

Periodontal regeneration can be defined as the complete restoration of lost periodontal tissues to their original architecture and function by recapitulating the crucial wound healing events associated with their development (14). Among the various surgical techniques used to achieve the ideal biologic conditions required for periodontal regeneration, open flap debridement (OFD) or access flap surgery was promising among the earlier procedures used (15,16). Conventional OFD resulted in significant clinical benefits when used along with various biomaterials (17–20). However, these approaches falls short of regenerating tissues destroyed by the disease, and have a limited potential towards attaining a complete periodontal regeneration (21).

This complete regeneration can only be initiated by activation of specifically periodontal ligament derived cells of remaining periodontium, as they possess the capability to differentiate into new fibroblasts, cementoblasts, and osteoblasts. This led to the concept of compartmentalization (22), where placement of a physiological barrier membrane would help to exclude the gingival connective tissue and epithelium from the healing process, and giving way for the PDL cells to repopulate the confined intra-osseous defect (23). Use of Guided tissue regeneration with various bone grafts is considered to be the gold standard procedure in treatment of intra-osseous defects. Despite these therapeutic procedures, bone fill in two or three walled defects can still be seen in histological sections with a long junctional epithelium attached to root surface, which does not represent true regeneration.

The concept of tissue engineering in achieving periodontal regeneration requires the presence of cells, scaffold and signaling molecules. Cells required for periodontal regeneration are found from the remaining periodontal structure. Use of blood clot, bone grafts and guided tissue regeneration serve the purpose of scaffold. But, there always exists a need of signaling molecules, which lack in such wound healing events.

1.1 ASSESSMENT OF THE DEFECT –

Clinically, an area of vertical bone loss often demonstrates deeper probing depths localized to one or more tooth surfaces. Despite the identified trends, vertical bone loss can also be associated with anterior teeth and are often amenable to periodontal regeneration.

Radiographs can provide insight regarding the defect configuration and severity and extent of its involvement with the root. Once an area of vertical bone loss has been identified, the characteristics of both the bone defects and the affected tooth must be assessed. The configuration of the defect aids in determining the predictability of regenerative therapy. Tooth related factors such as root form, mobility, extent of furcation involvement, and amount of remaining bone support are equally important (24).

1.2 EFFECT OF DEFECT MORPHOLOGY ON REGENERATIVE THERAPY PROGNOSIS

Defect morphology includes assessing for bone walls, number of bone walls and the depth and width of periodontal defects.

Periodontal osseous lesions has been broadly classified in to: **Goldman and Cohen 1958** (25)

1. Suprabony defects

2. Intrabony defects

3. Inter-radicular or furcation defects

4. Combined defects

Intra-bony defects / Vertical defects

Intrabony defect is an osseous defect with its base apical to the crest of alveolar bone. Vertical defects or angular defects are those that occur in an oblique direction leaving a hollowed out trough in the bone alongside the root.

According to the criteria presented by **Goldman and Cohen in 1958** (25), the intrabony defects are classified as:

One wall defect: Defects limited by one osseous wall and the tooth surface. (C)

Two wall defect: Defects limited by 2 osseous walls and the tooth surface. (B)

Three wall defect: Defects limited by 3 osseous walls and the tooth surface. (A)

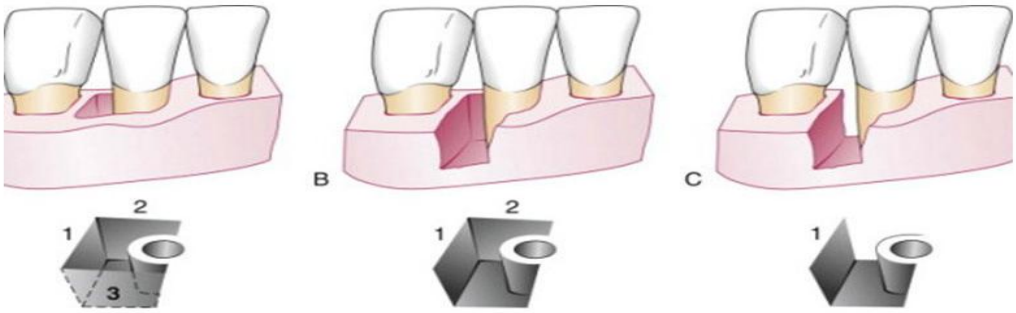

Figure 1. Vertical bone defects (Source: Carranza's Textbook of Clinical Periodontology, Saunders)

The bone wall of the defect provides physical support and containment of the regenerative materials. The bone walls can also be a source of osteoprogenitor cells as well as cells derived from periodontal ligament. In general three wall defects had 95% bone fill, compared to 82% for two wall defects and 39% fill for one wall defects (26).

Wide defects present a greater challenge for bone regeneration since the osteogenic cells must migrate farther. Conversely, a narrow defect would more effectively facilitate osteoblast migration and bone regeneration. A number of studies have evaluated periodontal defect width and its influence on bone regeneration which have consistently evaluated and showed a positive correlation between narrow, deep defect width (or a more acute angle defect) and bone fill (27)**.** The alveolar bone regeneration are almost totally restricted to locations where there was an angular bone defect (28).

1.3 REGENERATIVE PERIODONTAL TREATMENT FOR BONE DEFECTS –

Periodontal regeneration is defined as the reproduction or reconstitution of lost or injured tissue so that the form and function of the lost structures are restored. Regeneration of any tissue type in a complex biological process in itself, requiring intricately regulated interactions between cells, locally acting growth factors, systemic hormones, and the extracellular matrix components in which these interact. In periodontium, such regeneration involves the creation of new alveolar bone, cementum and periodontal ligament (29).

Several researchers have aimed at regeneration of periodontal tissues lost as a result of periodontal diseases. Some approaches such as bone grafting (30), guided tissue regeneration (GTR) using barrier membranes or emdogain application or even combinations of the above have been used with varying results. Open flap debridement (OFD) or access flap surgery was promising (31) when used alone or in adjunct to GTR, bone grafts and platelet concentrates.

1.4 OPEN FLAP DEBRIDEMENT –

The use of access flap surgery is recognised standard to manage residual pockets after cause related therapy. Recent systematic reviews shown that it results in shallower pockets and a modest increase in clinical attachment levels (32,33). In exploratory analysis, benefit seem to increase as residual pocket depths increase. In this context, the improvements in surrogate outcomes are significant, as they are observed in the studies at low risks of bias and thus at low risk of overestimation of the treatment effects and in challenging defects where the application and osseous resective approaches is limited by the compromise of the support of adjacent teeth. The use of specific biomaterials or biologic agents was more effective than OFD in improving attachment levels in intraosseous defects (34).

1.5 TISSUE ENGINEERING IN PERIODONTAL REGENERATION -

The presence of tissue engineering in achieving periodontal regeneration requires the presence of cells, scaffold and signalling molecules. Cells required for periodontal regeneration are found from the remaining periodontal structure. Uses of bone grafts and guided tissue regeneration serves the purpose of scaffold, but it lacks signalling molecules which are required for wound healing events.

Recently polypeptide growth factors, which are biologic mediators which regulates cell proliferation, chemotaxis and differentiation, were investigated. As preliminary evidence for their potential application in periodontal wound healing, several poly peptide growth factors have been identified in the human periodontal tissues by immunohistochemistry and in situ hybridization (35).

A new method in this field was usage of concentrated platelet products which are the source of autologous platelet derived growth factors and transforming growth factors (36).

1.6 PLATELET CONCENTRATES-

In 1974, platelets regenerative potential was introduced (37). After activation of the platelets which are trapped within fibrin matrix, growth factors released and stimulate the mitogenic response in the bone periosteum during normal wound healing for repair of the bone (38).

In transfusion medicine, platelet concentrates were originally used for the treatment and prevention of haemorrhage due to severe thrombopenia, which are often caused by acute leukemia or significant blood loss during long lasting surgery. The use of blood derived products to seal wound and stimulate healing started with the use of fibrin glues and are constituted of concentrated fibrinogen (polymerization induced by thrombin and calcium). Risk of cross infection from these commercial adhesives led to the development of autologous fibrin sealants from the patient's own plasma (39). However, their fabrication resulted in less reproducible or less satisfactory rheological properties and their use remained very limited owing to the complexity and the cost of their production protocols (40).

Platelet contain high quantities of key growth factors, such as PDGF-AB (platelet derived growth factor AB), TGF β1 (transforming growth factor β1) and VEGF (vascular endothelial growth factor), which are able to stimulate cell proliferation, matrix remodelling and angiogenesis. The uses of these growth factors are made possible by discovery of platelet rich plasma and platelet rich fibrin with advancement in a wide range of preparation protocols, kits and centrifuges.

1.7 PLATELETS – ORIGIN, MORPHOLOGY AND DISTRIBUTION

Platelets are cytoplasmic fragments of megakaryocytes (a type of white blood cell), formed in the bone marrow, round or oval in shape, approximately 2 μm in diameter (41). They have a trilaminar cell membrane with a glycoprotein receptor surface overlying and partially interspersed with and penetrating a bilayer of phospholipid and cholesterol. Platelets lack nuclei but contain organelles and structures such as mitochondria, microtubules, and granules (alpha, delta and lambda).

There are approximately 50 to 80 alpha granules per platelet, each bound by a unit membrane and formed during megakaryocyte maturation. The granules are approximately 200 to 500 nm in diameter

and contain over 30 bioactive proteins, many of which have a fundamental role in hemostasis and/or tissue healing. The platelet cytoplasm contains an open, canalicular system that increases the effective surface area for intake of stimulatory agonists and discharge of effectors' secretions. The sub-membrane region contains microfilaments of actin and myosin that mediate morphologic alterations. The normal concentration of platelets in blood is approximately 1,50,000 to 4,00,000 platelets/mm3. These remain in circulation for an average of approximately 10 days before removal by macrophages of reticulo-endothelial system.

Functionally, platelets are involved with both hemostasis and the initiation of wound healing.

Platelet role in hemostasis – After tissue injury, platelets become exposed to damaged blood vessels, which place them in direct contact with collagen, the basement membrane of capillaries, and sub epithelial micro fibrils. This interaction causes the platelets to aggregate at the site and change from a round shape to one that includes large, sticky protuberances, or pseudopodia. This process is called platelet activation. During activation, alpha granules fuse with the platelet plasma membrane and release their protein contents to its surrounding, for small vascular defects, this platelet plug may be sufficient to stop blood loss; however, if the defect is large, a blood clot may be required. Blood clotting is initiated by one of the two pathways, namely, the intrinsic and extrinsic pathways. Both pathways involve a cascaded reaction sequence whereby inactive factors gets activated which, in turn, catalyze the formation of other products from precursors that go on to catalyze subsequent reactions, leading to formation of a blood clot.

Platelet role in wound healing- Numerous proteins are contained within the alpha granules of platelets that strongly influence wound healing, including platelet derived growth factor (PDGF), transforming growth factors (TGF β), platelet factor 4 (PF4), interleukin 1 (IL1), platelet derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin like growth factor (IGF), epithelial cell growth factor (ECGF), steocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin (TSP). Collectively, these proteins are members of families of growth factors, cytokines and chemokines which are broadly referred to as secretory proteins.

Activation of platelet causes degranulation of alpha granules, and releasing active forms of these growth factors, and allowing them to bind to the transmembrane receptors of target cells (e.g., mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells). Intracellular signal protein gets activated, which results in the expression of the gene sequence that directs cellular proliferation, matrix formation, osteoid production, collagen synthesis and so forth.

1.8 ROLE OF VARIOUS GROWTH FACTORS

Platelet derived growth factors (PDGF) –

PDGF are glycoprotein with a molecular weight of approximately 30kd. Although it was described in the alpha granules of the platelets, and also synthesized and secreted by other cells, such as macrophages and endothelium. It seems to be the first growth factor present in a wound, and it initiates connective tissue healing, including bone regeneration and repair. In humans, it exists mostly as a heterodimer of two chains termed A and B chains – of about equal size and molecular weight. Homodimers of AA and BB chains are also present in human platelets and have same effects on bone regeneration.

PDGF is known to emerge from degranulating platelets at the time of injury. Its mechanisms were to activate cell membrane receptors on target cells, which in turn are thought to develop high energy phosphate bonds on internal cytoplasmic signal proteins. The bonds then activate the signal proteins to initiate a specific activity within target cells. The important specific activities of PDGF include mitogenesis (increase in the cell populations of healing cells), angiogenesis, macrophage activation and a second phase of growth factors for continued repair and bone regeneration. There are approximately 0.06 ng of PDGF per one million platelets, a fact that underscores this molecules great potency. At a higher concentration of PDGF, it was believed to be present more proximally in wound areas where cell migrations, neutrophil activation and collagenase release of fibroblast and cell division have occurred. PDGF thus plays a pivotal role in wound healing (42). PDGF stored in the bone matrix and released upon activation of osteoblasts, resulting in new bone formation (43). In support to these findings, in vitro study demonstrated that PDGF initially stimulates bone resorption

and also stimulates the proliferation and chemotaxis of osteoblasts (44). Therefore a greater concentration of platelets can be expected to have a profound effect on wound healing enhancement and bone regeneration.

Transforming growth factors (TGF)

TGF-β belongs to the superfamily of growth factors and differentiating factors of which the bone morphogenic protein (BMP) family, containing at least 13 described BMPs (36). The TGF βs are referred to and studied in previous articles are TGF β1 and TGF β2 proteins, which are involved in general connective tissue repair and bone regeneration. They are proteins that have molecular weights of approximately 25 kd. Like PDGF, they are synthesized and found in platelets and macrophages, as well as in some other cell types. When released by platelet degranulation or actively secreted by macrophages, they act as paracrine growth factors (i.e. growth factors secreted by one cell exerting its effect on the adjacent cell), affecting mainly fibroblast, marrow stem cells, and preosteoblasts.

The important function of TGF β1 and TGF β2 seems to be the chemotaxis and mitogenesis of fibroblasts, marrow stem cells, endothelial cells, epithelial cells, and pre-osteoblastic cells, and they have ability to stimulate osteoblasts deposition of collagen matrix of wound healing and of bone. In addition, TGF-βs inhibits osteoclast formation and bone resorption by stimulating chemotactic migration of osteoblast to the site of injury thus favouring bone formation over resorption (36).

TGF was also found at higher levels in bone matrix and upon activation facilities wound healing under inflammation conditions (45). TGF β1, when coated on beta tricalcium phosphate pellets, substantially stimulated cell proliferation and differentiation of osteoblasts lineage cells, and eventually induced new bone formation in experimental rat calvarial osseous defect (46). In addition, in association with gingival wound healing after flap surgery in rats, the topically applied TGFβ1 demonstrated that it stimulated the proliferation of gingival fibroblastic cells, the formation of blood vessels, and remodelling of extracellular matrix molecules, which resulted in increased formation of granulation tissue in periodontal healing (47).

1.9 EVOLUTION OF PLATELET CONCENTRATES –

Owing to the search for improved haemostatic agents and surgical adhesives, fibrin glue (alternatively referred to as fibrin sealant or fibrin gel) was developed in 1970. It is classically described as a two component mixture in which concentrated fibrinogen, factor XIII and fibronectin are added to thrombin, calcium chloride and an inhibitor of fibrinolysis to form a fibrin clot. The mechanism of action of fibrin adhesives reproduces the last stages of coagulation during which fibrinogen is converted into fibrin (48).

The short comings of this fibrin glue were risk of transmission of virus, like human immune-deficiency virus (HIV). Due to the increased risks associated with the use of fibrin glue, its marketing was prohibited and attempts at the development of autologous fibrin adhesives increased (49). In 1994, autologous fibrin adhesive was described, in which patient's blood is harvested 1-3 weeks before the intervention and requires separating one unit of whole blood into red blood cell component and the plasma fraction for use as a cryoprecipitate which thawed over 24 hours before being ready to use (50).

Limitations–

- Extremely long and complex protocol
- Potential transfusion reaction and infectious disease complication
- Patients have to meet the blood bank's criteria for weight, haemoglobin concentration, age and general health status. Failure to do so disqualifies patient from its usage.

Consequently, to overcome these problems and to boost intrinsic characteristics of fibrin sealants, the use of platelet concentrates, has been considerably increased during the last decade (49).

1.10 CLASSIFICATION OF PLATELET CONCENTRATES (51) **–**

L-PRP – Leucocyte and platelet rich plasma

P-PRP – Pure platelet rich plasma

L-PRF – Leucocyte and platelet rich fibrin

P-PRF – Pure platelet rich fibrin

1.10.1 FIRST GENERATION PLATELET CONCENTRATES

The use of autologous products with high platelet concentration such as Platelet rich plasma (PRP), Platelet concentrates (PC) and platelet gels developed to combine the fibrin sealant properties with the growth factor (GFs) effects of platelets – providing an ideal growth factor delivery system at the site of injury. The scientific rationale behind the use of these preparations lies in the fact that growth factors are known to play a crucial role in hard and soft tissue repair mechanisms (36,52). These GFs exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, regeneration and cell proliferation. Platelet rich plasma (PRP) contains 4% red blood cells, 95% platelets and 1% white blood cells (53).

ADVANTAGES OF PLATELET GEL AND PRP OVER FIBRIN SEALANT – (49)

- Safe autogenous preparation, free from concerns over transmissible disease such as HIV, hepatitis.
- Convenient for patient since blood is collected in the immediate postoperative period.
- More patients are eligible for this procedure because the criteria of blood bank donation do not have to be met, thus including elderly whose medical conditions would preclude the blood bank from drawing a whole unit of blood.
- Presence of platelets brings cytokines and growth factors to the site of surgery in a manner that would not occur in fibrin glue.

CLINICAL APPLICATION OF PRP (54)

- In periodontal bone defects (intrabony and soft tissue defects)
- In sinus lift procedures
- Ridge augmentation
- Oral/nasal fistula repair
- Jaw reconstruction surgeries
- Soft tissue surgeries like gingival grafts, sub epithelial grafts because of its property of increasing soft tissue healing.

Potential risk of using PRP (55)

- Concern over the use of bovine thrombin, bovine factor Va may be a contaminant in certain bovine bone thrombin commercial preparations, antibodies to bovine factor Va may cross react with human factor V a and may produce coagulopathies and rare bleeding episodes
- Lack of uniformity in PRP preparation
- Release of growth factors for short period of time.

1.10.2 SECOND GENERATION PLATELET CONCENTRATE -

PRF was first developed in France in 2001 (56). This second generation platelet concentrate eliminates the risk associated with the use of bovine thrombin (57). The protocol for procuring PRF is very simple. Blood sample is taken from without anticoagulant in 10 ml tube which is immediately centrifuged in a table centrifuge at 3000rpm (approximately 400g) for 10 minutes (58).

Approximately 97% of the platelets and >50% of the leukocyte were concentrated in the PRF clot and showed a specific 3-dimentional distribution, depending on the centrifugation forces (59). The absence of anticoagulant implies the activation in a few minutes of most platelets in contact with the glass tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the higher part of the tube before the circulating thrombin transforms into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma

at the top. The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. Quick handling is the only way to obtain a clinically usable PRF clot (60). PRF is a natural bioactive membrane, which can enhance soft/hard tissue healing, at the same time, can also protect surgical sites (61)

Properties of PRF –

- PRF consists of an intimate assemble of cytokines, structural glycoproteins enmeshed within a slowly polymerized fibrin network. The biochemical components have well known synergetic effects on healing processes (58)
- PRF is not only a platelet concentrate but also an immune node able to stimulate defence mechanisms. It is likely that the significant inflammatory regulation noted on surgical sites treated with PRF is the outcome of retro control effects from cytokine strapped in the fibrin network and released during the remodelling of this initial matrix (62)
- Fibrin matrix present in PRF act as a natural guide of angiogenesis, also helps in wound coverage (63).

CLINICAL IMPLICATION OF PRF

- Sinus lift procedures (64)
- Used for gingival recession coverage procedure (65)
- Socket preservation (66)
- Periodontal intrabony and furcation defects (67)
- Soft tissue augmentation (65)

ADVANTAGES OF PRF –

- No need of addition of bovine thrombin and other anticoagulants (60)
- Slow release of PRF compared to PRP and hence better healing properties (56)
- Better organized fibrin matrix, hence able to efficiently direct stem cell migration and the healing program (68)

LIMITATIONS OF PRF –

- Only limited volume of PRF can be used. Because it is obtained from an autologous blood sample, the quantities produced are low (60).
- Quick handling is required immediately after collection. The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. In fact, without anticoagulant, the blood sample starts to coagulate almost immediately upon contact with the tube glass. Quick handling is the only way to obtain a clinically usable PRF clot (64).
- The fibrin matrix contains all the circulating immune cells and highly antigenic molecules. That is why PRF membranes are totally specific to the donor and cannot constitute an allogenic graft tissue (57).

1.11 PLASMA RICH IN GROWTH FACTORS (PRGF) or PURE PLATELET RICH PLASMA–

Plasma Rich in Growth Factors (PRGF®) is a mixture of autologous proteins, prepared from a certain volume of platelet-rich plasma obtained from a small volume of blood, which does not contain leukocytes. It is prepared by a single centrifugation step, separating the plasma into two fractions with increasing concentrations of platelets and growth factors. The more superficial fraction contains a number of platelets similar to the peripheral blood and can be used for the preparation of fibrin membranes. The fraction closer to the red cells is the richest in platelet growth factors. The advantages of this protocol respect to others are multiple, since it can be used in a clinic with a simple equipment. In addition, the discomfort for the patient and the possibilities of complications are minimal due to the small volume of blood drawn, which does not imply any alteration in the blood parameters. The patient's own blood is used without the need to mix it with allogenic or xenogenic hemo-derivatives, which may have the slightest antigenic or contagious effect. In recent years, the research done and the clinical use of PRGF® has led to the publication of several scientific articles related to in vitro and animal studies and to various clinical trials in the field of oral and maxillofacial surgery.

In 1999, the concept of PRGF technology evolved for the first time. The term PRGF identifies exclusively 100% autologous and biocompatible formulations elaborated by a one – step centrifugation process using sodium citrate and calcium chloride as anticoagulant and activator respectively. Plasma rich in growth factors has moderated platelet concentration and does not contain leucocytes, with the aim of avoiding the pro-inflammatory effects of proteases and acid hydrolases in white blood cells (69).

PRGF offers numerous advantages:

- It allows simultaneous action of multiple growth factors, and is an autologous product.
- PRGF also increase tissue vascularization and it is biocompatible, safe, and is resorbed by the body within days after initiating local regeneration (70).
- Also exogenous bovine thrombin is not used as an activator of PRGF thus avoiding the risk of immunological reactions and disease transmissions (71).

PREPARATION OF PRGF –

Blood drawn from the patient and collected with 3.8% trisodium citrate as anticoagulant is used. The glass tube containing the blood is subjected to centrifuge at 580G for 8 minutes. As a result, plasma gets separated into different fractions:

1. Red blood cells at the bottom of the tube
2. Plasma poor in growth factors at the top of the tube
3. Plasma rich in growth factors in the middle of the tube just above the settled red blood cells.

The fraction located immediately above the erythrocytes is collected from the each tube and transferred into sterile tubes. Activation of Plasma is done by adding 10% of calcium chloride. 50µl of 10 % calcium chloride is added to sterile tube containing 1ml of PRGF. Following 5-7 minutes at room temperature or 2-3 minutes at 37^0c (in heat block), a PRGF gel is formed.

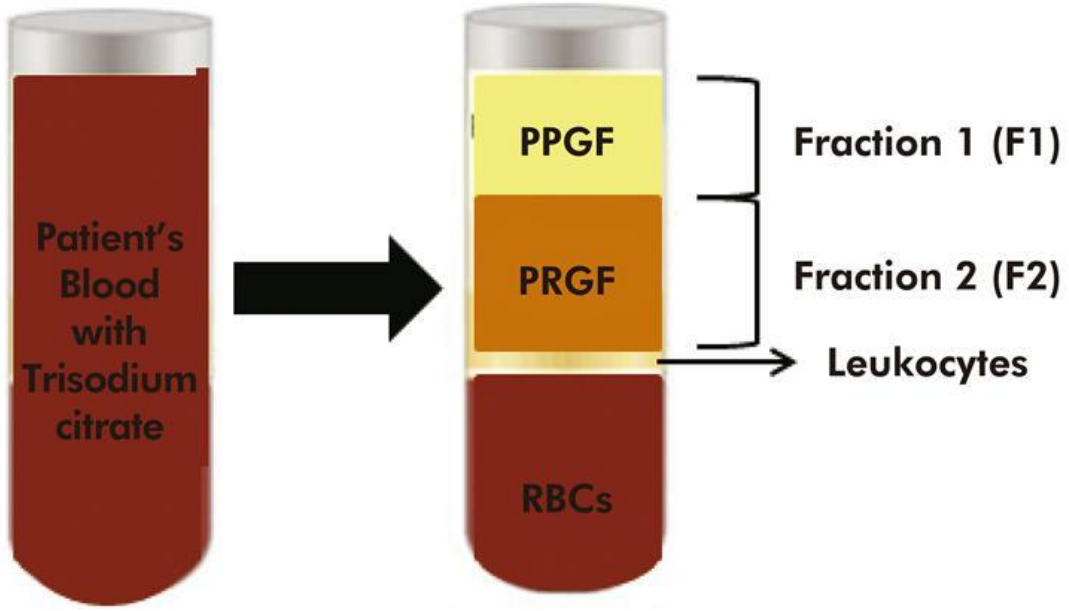

Figure 2. Diagrammatic representation of PRGF layer

The specificity of this technique is that the buffy coat layer (between the red blood cells base and the acellular plasma) is not harvested in order to avoid the collection of leukocytes. Since the buffy coat layer contains most leucocytes and also many platelets, the final PRGF is a plasma suspension with almost no leukocytes and a lower platelet concentration than other PRPs.

Also addition of calcium chloride promotes the for formation of native thrombin , mimicking the physiological process and enabling a more sustained release of growth factors, which might be crucial to proper tissue repair and wound healing. Moreover, this procedure obviates immunological reactions and the risk of the disease transmission associated with the use of exogenous bovine thrombin.

PRGF contains moderately elevated platelet concentration of ~6 x 10^5 platelets /µl, which has been reported to induce the optimal biological benefit. In fact lower concentration can lead to suboptimal effect and, whereas higher concentration might have an inhibitory effect. Also the absence of leucocytes in PRGF improves the homogeneity of the product, and thus prevents the destruction of surrounding cells at the healing sites by the reactive oxygen species and MMPs produced by the neutrophils (72).

Invitro effect of PRGF-

Comparing the in-vitro effect of PRGF with PRF on human gingival fibroblasts, PRGF showed positive effect in 24, 48, 72 hours whereas PRF showed positive effect only upto 24 hours after which it had a negative effect and caused by 38% and 60% decrease in viability and proliferation on the human gingival fibroblasts proliferation. Thus PRGF strong stimulatory effect on human gingival fibroblast than PRF (73)**.**

Mechanical stiffness of PRGF was equal to that of L-PRF when the tensile loads were applied but L-PRF showed a better tensile strength and toughness than PRGF (74)**.**

While the amount of leucocyte content is higher in PRP and PRF, it is almost nil in PRGF thus improves the immune activity of leucocytes at the surgical sites and also increases the homogeneity of the product (75). Addition of leukocytes may increase the inflammatory response at the site due to the metalloproteases secreted by leukocytes and proinflammatory proteases and acid hydrolases contained in white blood cells (76). Furthermore the enzymes released may also induce a faster degradation of the supporting fibrin scaffold which acts as growth factors delivery system and a provisional cell matrix. PRGF has a strong inhibitory effect on S.Aureus and S.Epidermis and thus showing its antibacterial effect (77).

In bone defects –

PRGF has a positive effect when combined along with deproteinized bovine bone in rabbit calvarial bone defects within a time interval of 8 weeks defects (78), thus enhancing the process of osteogenesis. PRGF showed similar effects when tibial bone defects in rabbits thus they also help in local bone formation (79).

PRGF also exerts a positive effect in peri-implant bone healing. They also help in increasing the trabecular thickness and enhances bone maturity (80).

When used in human extraction socket PRGF had no short term effects but showed long term effects in bone healing (81).

In periodontal bone defects –

Numerous positive effects of PRGF was reported when used in human periodontal defects. PRGF when placed along with autologous bone grafts in grade 2 furcation defects lead to significant improvement in vertical and horizontal attachment level, also decreases the vertical depth and horizontal extent of the defect (82).

PRGF also exerted similar effect when used along with bovine porous bone mineral in grade 2 furcation defects (83).

In soft tissue healing –

Not only pertaining to hard tissue but PRGF also shows positive results when used along with sub-epithelial connective tissue grafts for treating gingival recession (84).

Despite the growing number of publications in the literature regarding the use of PRGF in the field of oral and maxillofacial surgery and periodontics, there are still few published randomized clinical trials concerning the use of PRGF in the regeneration of periodontal intra bony defects. The present clinical trial was designed with the objective of evaluating the clinical and radiological efficacy of Plasma Rich in Growth Factors (PRGF ®) for the regeneration of periodontal intra bony defects.

1.12 INTRA- MARROW PENETRATION (DECORTICATION):

Intra-marrow penetration (IMP), also known as decortication, serves as a means to improve the local blood vessel and progenitor cell supply and, consequently, the outcomes of surgical procedures used to treat intra-bony defects (85). IMP accelerate initial bone neogenesis results in increased bone fill and density suggesting that its use can be beneficial in bone regeneration procedure (86). It favors clot formation and maturation, which is considered a key factor during periodontal regeneration. IMP also promotes increased expression of RANKL (receptor activator of the nuclear factor-kB ligand), which

is a significant element of the process of regeneration (87). This procedure is inexpensive, can be done in approximately 1 min and hence no cost for bone graft to the patients.

In the literature, IMP is widely used as part of a regenerative procedure to treat intra-bony defects. IMP is applied either at the discretion of the surgeon or only when there was no bleeding in the defect or in all defects. Predictable bone regeneration involves restoring lost supporting structures of the dentition such as cementum, periodontal ligament and bone to the disease root surface. A study (8) have demonstrated that addition of IMP to an open flap debridement procedure used to treat intra-bony defects results in statistically and clinically significant enhancement of both clinical and radiographic outcomes.

CHAPTER 2. AIM & OBJECTIVES

The aim of the study was to evaluate the clinical and radiological efficacy of PRGF in adjunct to OFD compared to OFD alone for management of periodontal intra-osseous defects in periodontitis patients.

Objectives:

1. To evaluate the improvement of clinical and radiological parameters with use of PRGF in adjunct to OFD for management of periodontal intra-osseous defects in periodontitis patients.
2. To evaluate the improvement of clinical and radiological parameters with use of OFD alone for management of periodontal intra-osseous defects in periodontitis patients.
3. To compare the clinical and radiological parameters between PRGF in adjunct to OFD compared to OFD alone for management of periodontal intra-osseous defects in periodontitis patients.
4. To compare the patient reported outcomes between PRGF in adjunct to OFD compared to OFD alone for management of periodontal intra-osseous defects in periodontitis patients.

CHAPTER 3. MATERIALS AND METHODS

The study was conducted at Siksha 'O' Anusandhan (Deemed to be) University as a part of dissertation to be submitted for University of Milan, between June 2019 to September 2020. The study protocol was approved by the Scientific Review Board and Institutional ethical committee of Siksha 'O' Anusandhan (Deemed to be) University. Forty contra-lateral sites from twenty subjects, diagnosed with intra-bony defect (IBD), were recruited from the Department of Periodontics and Oral Implantology based on the following criteria.

3.1. INCLUSION CRITERIA

1. Subjects within the age group of 30 years to 50 years.
2. Subjects diagnosed with GRADE A/B and STAGE II/III Periodontitis (Classification of Periodontal and Peri-implant Conditions by 2017 World workshop of AAP and EFP)
3. Presence of 2-walled or 3-walled inter-proximal IBDs ≥ 3mm deep (distance between alveolar crest and base of the defect)
4. Presence of inter-proximal probing depth(PD) ≥ 5mm following phase I therapy (SRP)
5. Systemically healthy subjects with no debilitating conditions.

3.2. EXCLUSION CRITERIA

1. Subjects with history of periodontal surgical treatment within the last six months.
2. Former / current smokers.
3. Immuno-comprimised subjects.
4. Subjects having unacceptable oral hygiene (PI >1.5) after re-evaluation of phase I therapy.
5. Pregnant and lactating mothers.
6. Subjects on antibiotic therapy within the last six months.
7. Subjects on treatment with steroids.
8. Subjects with non-vital teeth, teeth with furcation defects or teeth with mobility ≥ Grade II and teeth with endodontic-periodontal involvement.
9. Acute abscesses of periodontium.

3.3. STUDY DESIGN

The study conducted was a randomized controlled trial, with a split-mouth design, comparing the use of Plasma Rich in Growth Factors (PRGF) with Open flap debridement (OFD) and decortication (intra-marrow penetration) as experimental group and Open flap debridement (OFD) with decortication (intra-marrow penetration) as control group, in treatment of periodontal intra-bony defects.

3.4 STUDY GROUPS

Group 1- Treated with use of Plasma Rich in Growth Factors (PRGF) with Open flap debridement (OFD) and decortication (intra-marrow penetration)

Group 2- Treated with use of Open flap debridement (OFD) with decortication (intra-marrow penetration)

3.5 SAMPLE SIZE CALCULATION

To calculate the sample size to compare two proportions is given by

$$n = \frac{\left(Z_\alpha + Z_\beta\right)^2 (s_1^2 + s_2^2)}{(M_1 - M_2)^2}$$

This n is sample size per group.

Where Z_α: Z Value for level of significance: 1.96 (5% level of significance)

Z_β: Z Value for power of the test:1.282 (power 90%)

According to the pilot study conducted with similar groups with 8 sites in 4 patients, the mean difference observed for primary outcome intra-bony defect depth (IBDD) was:

$M_1 – M_2$: Difference between two means: 1.89

s_1: Standard deviation of sample 1: 1.75

s_2: Standard deviation of sample 2: 1.52

By substituting these values in the above formula give the required sample size.

$$n = \frac{(Z_\alpha + Z_\beta)^2 (s_1^2 + s_2^2)}{(M_1 - M_2)^2}$$

$$n = \frac{(1.96 + 1.282)^2 * (1.75^2 + 1.52^2)}{(1.89)^2}$$

$$n = \frac{(3.242)^2 * (3.0625 + 2.3104)}{3.5721}$$

$$n = \frac{(10.51) * (5.3729)}{3.5721}$$

n= 15.8 (16 per group)

The required sample size was estimated to be 16 per group, a total of thirty two sites, under 80% power, with level of significance set at 5%.

3.6 STUDY PATIENTS

A total of 26 patients with 52 sites, suffering from GRADE A/B and STAGE II/III Periodontitis, were selected based on the selection criteria mentioned above.

All subjects were subjected to Phase 1 therapy such as scaling and root planing for the purpose of pre-surgical preparation protocol. 1 week after phase1 therapy, re-evaluation was done to ensure the fitness of the subject to undergo the surgical phase. After evaluation, only 20 patients with 40 contra-lateral sites were recruited for this trial. The reason for exclusion of rest 6 patients are provided in the consort flow diagram.

3.7 PARAMETERS ASSESSED

The following clinical, radiological and patient reported parameters were assessed at baseline and different time-points. The baseline measurements were made after 1 month follow visit of complete scaling and root planing. The details of each parameter and their methods of assessment are described below:

3.7.1 Clinical parameters:

1. Plaque Index (FMPI/SSPI)- Silness and Loe 1964
2. Gingival index (SSGI) – Loe and Silness 1963
3. Gingival Marginal Level (GML)
4. Probing Pocket Depth (PPD)
5. Relative Attachment Level (RAL)
6. Bleeding on Probing (SSBOP)

1. Plaque Index –FMPI and SSPI - (Silness and Loe, 1964)

Recordings were made using a mouth mirror and a dental explorer, after air drying of the teeth and gingiva to assess plaque.

Scoring Criteria:

Score 0- No plaque in the gingival area

Score 1- A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface.

Score 2- Moderate accumulation of soft deposits within the gingival margin, which can be seen by the naked eye

Score 3- Abundance of soft matter within the gingival pocket and/or on the gingival margin.

$$\text{Plaque index for individual tooth} = \frac{\text{Total score (4 areas of the tooth)}}{4}$$

Plaque score for a tooth was obtained by adding four values per tooth and dividing by four. Scores of each tooth are added and then divided by number of teeth examined to give plaque scores for the individual.

Ratings for plaque index:

Rating	Scores
Excellent	0
Good	0.1 - 0.9
Fair	1.0 - 1.9
Poor	2.0 – 3.0

2. Gingival Index- SSGI (Loe and Silness 1963)

Determines the severity of gingival bleeding, a sign of inflammation that is associated with periodontal disease. The gingival index was recorded at site-specific level.

A UNC-15 probe was used and passed along the gingival margin to provoke bleeding, and the clinical findings are recorded according to the following scores and criteria:

Score Criteria

Score 0 - No bleeding when a periodontal probe is passed along the gingival margin.

Score 1 - Isolated bleeding spots visible.

Score 2 - Bloods forms a confluent red line on gingival margin.

Score 3 - Heavy and profuse bleeding.

3. Gingival Marginal Level (GML) in mm:

Recorded by measuring the distance between the gingival margin and CEJ of the tooth with the help of a UNC-15 probe.

GML = Distance of GM to CEJ of tooth.

4. Probing Pocket Depth (PPD) in mm:

Measured using UNC-15 probe from the crest of gingival margin to the base of the pocket.

Standardization of PPD measurement:

All measurements were recorded using University of North Carolina periodontal probe with markings from 0 to15 with bands at 5, 10 and 15mm.

The measurements were standardized using customized acrylic stents, which were prepared on the study model of the subjects.

The occlusal stents for vertical probing were made using self cure acrylic resin, covering the occlusal as well as coronal 1/3rd of the buccal and lingual surfaces of the tooth involved and one tooth mesial and distal to involved tooth. The stents were trimmed flat on the bottom edge. The bottom edge of the stent serves the purpose of a Fixed Reference Point (FRP) for all the clinical measurements. Hemi-cylindrical vertical locating grooves were made on the buccal aspect of the stent with burs to guide the probe penetration vertically in the same plane every time it was inserted for recording the measurements. The customized acrylic stents were stored on the prepared study casts and immersed into water to minimize distortion for recording the clinical parameters at recall intervals postoperatively.

The following calculation of PPD was made from the clinical measurements recorded:

Probing Pocket Depth = (FRP to BP) – (FRP to GM)

Where, FRP = Fixed Reference Point

BP = Base of the pocket

GM = Gingival Margin

5. Relative Attachment Level (RAL) in mm:

Measured using UNC-15 probe from the lower border of acrylic stent to base of the pocket.

Standardization of RAL measurement:

The customized acrylic stent was placed on the selected teeth and the UNC-15 probe was gently inserted along the groove to the base of the pocket. The following calculation of RAL was made from the clinical measurements recorded:

Relative Attachment Level = (FRP to BP)

Where, FRP = Fixed Reference Point

BP = Base of the pocket

CEJ = Cemento-enamel junction

6. Bleeding on Probing (SSBOP):

The presence and absence of BOP was recorded at specific site. The probe was walked through the pocket and bleeding around the sulcus was noted at the end of 30 seconds.

3.7.2 Radiological parameters:

1. **Intra-bony Defect Depth (IBDD) in mm:**

Intraoral peri-apical radiographs (IOPA's) were taken using Long cone paralleling technique for site of intra-bony defect. 8 inch position indicating Film holders were used to secure the x-ray films in place. Individually customized bite blocks using putty index were made and stored for recording the IOPA's every time at same position at recall intervals post-operatively.

To ensure standardization in exposure of these radiographs, the exposure time was set for 0.8 seconds under voltage of 70Kv and 8mA current. It was ensured that there was no overlapping of image at interproximal areas of the tooth using paralleling technique. All the radiographs obtained were digitalized by using a 800 dpi scanner (HP Scanjet 3c/I, Hewlett Packard, USA). The digitalized radiographs were subjected to linear measurement for defect depth as follows:

IBDD = Distance from (the line joining CEJ of two adjacent teeth) to (Deepest point of the defect)

2. **Intra-bony Defect Area (IBDA) in mm2:**

The IBDA was calculated using IMAGE J (ImAGE, India) software, assessing the area formed by the intra-bony walls of defects in a 2-dimensional view of digitalized IOPA radiograph. The calculated area was expressed in mm^2.

3. **Percentage of IBDA fill (%IBDAF):**

Was calculated as follows:

% IBDAF = IBDA at baseline – IBDA at 6/9 months / IBDA at baseline * 100

3.7.3 Patient reported outcomes:

The Level of Pain experienced in first 7 days post-operatively measured by Visual Analogue Scale ranging from 0 to 100. Where, 0 corresponds to No pain and 100 corresponds to severe pain.

The following etiquettes were recorded from Day 1 to Day 7 by asking the patients to rate them from 0-5 (0- Never, 1- Almost Never, 2-Occasionally, 3- Quite Often, 4-Very Often)

1. Bleeding in region treated;
2. Swelling in region treated.

3.8 STUDY FOLLOW-UP DURATION

The total study follow-up duration was of 9 months.

All the clinical and radiological parameters were recorded and calculated at baseline, 3 months, 6 months, and 9 months.

3.9 RANDOMIZATION & ALLOCATION CONCEALMENT

The contra-lateral sites of each patient were randomized using coin toss method. The group allocated to the sites were sealed in an opaque envelope, which was opened at the time of surgery after debridement and decortication (intra-marrow penetration).

3.10 BLINDING

The study was double blinded, where the patients and the examiner evaluating all the parameters at various time points were masked.

3.11 SURGICAL PROCEDURE

The subjects were made to rinse with 0.2% chlorhexidine digluconate mouth rinse (Hexidine, ICPA pharma, India) for 30 seconds prior to the surgery. The defect sites were anaesthetized using 2% lignocaine (Lignox, Warren pharma, India) using block and infiltration techniques.

Sulcular incisions were given on the facial and lingual sides using bard parker knife with blade no. 15. (Hu-friedy, USA) Following this, a single vertical incision was made one tooth distal to the defect site, extending up to muco-gingival junction, keeping the base of the flap wider. A triangular, full

thickness muco-periosteal flap was raised to provide access to the defect. The defect was debrided off the granulation tissue and thorough root planing was carried out using curettes (Standard Graceys. Hu-freidy, USA). The surgical site was then irrigated with normal saline and carefully inspected for any remaining granulation tissue or deposits. Any adherent granulation tissue was trimmed from the under-surface of the flaps. The surgical procedure was carried out by an experienced surgeon who was masked to allocated groups.

3.11.1 FOR PRGF GROUP:

Intra-marrow penetration (Decortication):

The infra-bony defect cortical walls were penetrated using a round carbide bur #313 of 1-mm diameter (S.S White, SKU#313) to reach the marrow space: multiple perforations were performed not closer than 1 mm from each other and deep enough to obtain bleeding from the spongiosa.

Preparation of PRGF:

Blood was drawn from the patient and stored in 3.8% trisodium citrate as anticoagulant. The glass tubes containing the blood were centrifuged by digital machine at 580G for 8 minutes. As a result, plasma was separated into different fractions:

1. Red blood cells at the bottom of the tube
2. Plasma poor in growth factors at the top of the tube
3. Plasma rich in growth factors in the middle of the tube just above the settled red blood cells.

The fraction located immediately above the erythrocytes was collected from the each tube and transferred into sterile tubes. Activation of Plasma was done by adding 10% of calcium chloride. 50µl of 10 % calcium chloride was added to sterile tube containing 1ml of PRGF. Following 5-7 minutes at room temperature or 2-3 minutes at 37^0c (in heat block), a PRGF gel was formed.

Placement of PRGF into defect site:

The obtained PRGF gel was then packed in to the intrabony defect and condensed properly, so that the whole defect gets filled with the PRGF gel.

3.11.2 FOR OFD GROUP

Following degranulation and debridement of the defect site, the infra-bony defect cortical walls were penetrated using a round carbide bur #313 of 1-mm diameter (S.S White, SKU#313) to reach the marrow space: multiple perforations were performed not closer than 1 mm from each other and deep enough to obtain bleeding from the spongiosa.

Flaps were then repositioned and secured in place using (4-0) black braided silk suture (Ethicon). Sling and interrupted sutures were placed to obtain primary closure. The surgical sites were protected with a non-eugenol periodontal dressing (COE-PAK^{TM}).

Suitable antibiotics and analgesics (Amoxicillin 500mg thrice a day for 5 days and Paracetemol 500mg thrice a day for 3 days) were prescribed. Post-operative instructions were given to all the subjects and were instructed to report back after 7 days for suture removal.

3.12 POST-OPERATIVE EVALUATION

All clinical parameters were recorded at the end of 3, 6 and 9 months for all enrolled subjects. Radiological evaluation was carried out at the end of 6 and 9 month follow up using mentioned standardized protocols. The patient reported outcomes were recorded on all 7 days post –surgery by providing an recording template sheet to all patients.

3.13 STATISTICAL ANALYSIS

The data was collected and analysed using SPSS software version 26.0 (IBM, New-york, United States). The data was checked and found to follow normal or non-normal distribution using normality tests like Kolmogorov-smirnov test and Shapiro- wilk test. Therefore to analyze the data, parametric or non-parametric tests were applied, based on assessment of normality.

CHAPTER 4. RESULTS

This split-mouth, double blinded, randomized clinical trial was conducted on 20 patients with generalized periodontitis and presence of periodontal pocket in the contra-lateral sites with pocket depth measuring ≥5mm with an intra-bony defect component of ≥3mm. The sites were treated with two treatment strategies. One site served as control group, which was treated with OFD and decortications. And the other served as experimental group, which was treated by application of PRGF along with OFD and decortications.

The patients reporting to the Out-patient department of Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Bhubaneswar, were screened for eligibility. The eligible patients were subjected to Phase I scaling and root planing (SRP). The patients were asked to report after 1 month and baseline clinical and radiological parameters were recorded. The patients presenting with ≥5mm pocket depth at the 1 month visit after Phase 1 were recruited into this trial. All the clinical and radiological parameters were recorded at baseline. The patient reported outcomes were recorded after the surgical procedure for 7 consecutive days.

All the patients were followed up till the end of 6 months. However, only 12 patients completed the 9 months follow up. The reason of loss to follow-up was due to the COVID 19 pandemic, which led to lockdown and migration of patients to their respective home towns. No complications or adverse events were recorded.

The normality of the data was checked using Kolmogorov-smirnov test and Shapiro- wilk test.

Table 1. Demographic characteristics of recruited patients

Patient	GENDER	AGE	Walled Defect	Smoker	Teeth treated
01	M	42	3	Y	16/27
02	M	45	3	Y	14/25
03	F	51	Combined	N	15/25
04	F	53	3	N	16/27
05	F	50	3	N	17/27
06	F	41	Combined	N	16/26

07	F	56	3	N	17/26
08	F	52	3	N	35/46
09	F	46	3	N	36/46
10	F	62	Combined	N	16/26
11	M	66	3	Y	37/46
12	F	45	3	N	36/46
13	M	67	3	Y	15/26
14	M	68	2	Y	16/26
15	F	45	3	N	35/45
16	F	57	Combined	N	35/47
17	F	58	2	N	34/45
18	M	67	3	N	36/46
19	M	65	2	N	35/47
20	M	53	2	N	16/27

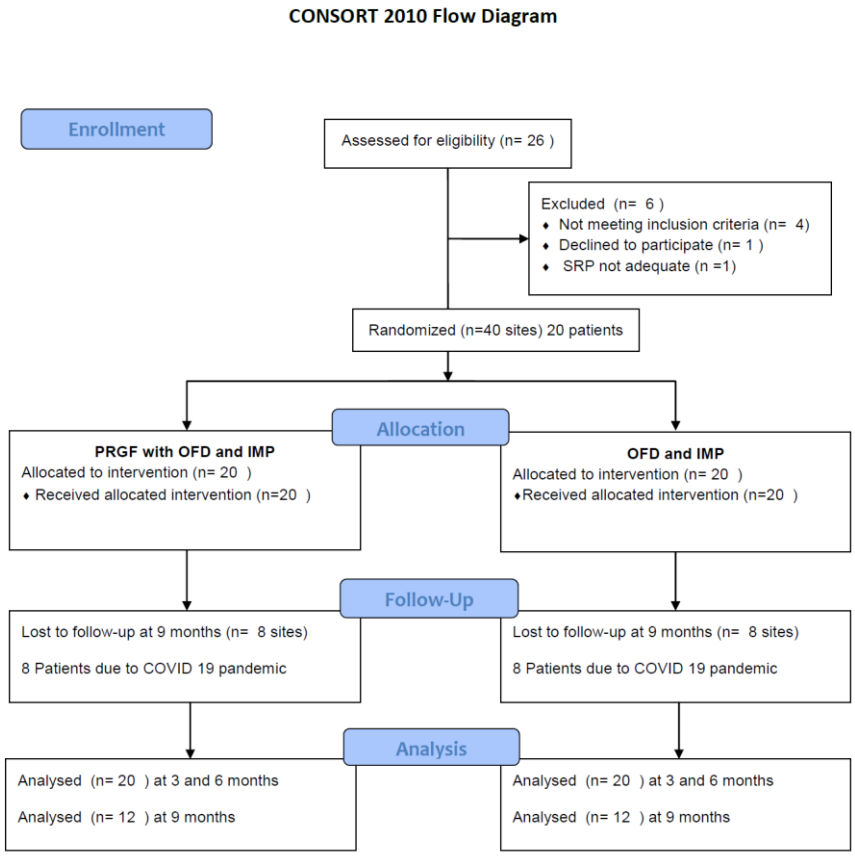

Figure 3: CONSORT FLOW DIAGRAM OF PATIENT SELECTION

4.1 Clinical indices

4.1.1 Full-mouth plaque index (FMPI) and Site-specific plaque index (SSPI)

The plaque index was assessed both in full-mouth and site specific level. FMPI showed significant improvement with time ($p<0.0001$) so as SSPI ($p<0.0001$). No statistical difference was observed in SSPI between the groups at any time-points suggesting similar efforts by patients in maintenance of oral hygiene at contra-lateral sites. (Table 1)

Table 1: Full mouth and Site specific plaque index

Groups		FMPI baseline	FMPI 3months	FMPI 6months	FMPI 9months	p-value (time)	SSPI baseline	SSPI 3months	SSPI 6months	SSPI 9months	p-value (time)
OFD	N	20	20	20	12		20	20	20	12	
	Mean	0.8005	0.6220	0.5392	0.5165	0.000[c*]	0.8978	0.6823	0.5510	0.4858	0.000[c*]
	SD	0.24165	0.15121	0.15084	0.20255		0.28470	0.16548	0.17465	0.22322	
PRGF	N	20	20	20	12		20	20	20	12	
	Mean	0.8005	0.6220	0.5392	0.5165	0.000[c*]	0.8135	0.6412	0.5096	0.4747	0.000[c*]
	SD	0.24165	0.15121	0.15084	0.20255		0.26672	0.18685	0.13315	0.18132	
P-value		1.000 [a]	1.000 [a]	1.000[b]	1.000 [b]		0.407 [a]	0.410 [a]	0.404 [b]	0.894 [b]	

a: Mann-Whitney test
b: student test
c: Freidman test
*(upto 6 months or upto 9 months)
FMPI- Full-mouth plaque index
SSPI- Site specific plaque index

4.1.2 Site-specific Gingival Index (SSGI) and Site-specific Bleeding on Probing (SSBOP)

The gingival index and bleeding on probing was recorded at site-specific level. The gingival index was corresponding to the recorded plaque index. And it was found to be significantly lower at the end of 9 months follow-up than baseline, 3 months and 6 months measurement for both groups. No significant differences were observed in SSPI between both groups at any time points. (Table 2)

However, interesting results were observed for SSBOP, where, SSBOP was found significantly lower for PRGF treated group than OFD treated group at the end of 6 months (p=0.041). But, similar trend was not observed at 9 months follow-up and the observation was found to be non-significant (p=0.140).

Table 2: Site-specific Gingival Index (SSGI) and Site-specific Bleeding on Probing (SSBOP)

Groups		SSGI baseline	SSGI 3months	SSGI 6months	SSGI 9months	p-value (time)	SSBOP baseline	SSBOP 3months	SSBOP 6months	SSBOP 9months	p-value (time)
OFD	N	20	20	20	12	0.000[c$] 0.015[c#]	20	20	20	12	0.000[c*]
	Mean	1.2500	0.8000	0.6500	0.4167		1.0000	1.0000	0.4500	0.3333	
	SD	0.71635	0.61559	0.48936	0.51493		0.00000	0.00000	0.51042	0.49237	
PRGF	N	20	20	20	12	0.000[c*]	20	20	20	12	0.000[c*]
	Mean	1.7500	,9500	0.7500	0.4167		1.0000	1.0000	0.1500	0.0833	
	SD	0.44426	0.22361	0.44426	0.51493		0.00000	0.00000	0.36635	0.28868	
p-value		0.017[a]	0.249 [a]	0.496 [a]	1.000 [a]		NS	NS	0.041[a]	0.140 [a]	

a: Mann-Whitney test
b: student test
c: Freidman test
*(upto 6 months or upto 9 months)
# upto 6 months
$ upto 9 months
SSGI – Site-specific Gingival Index
SSBOP- Site specific bleeding on probing

4.2.Clinical parameters

4.2.1 Probing pocket depth (PPD)

Intra-group comparison within all time points showed statistically high significant differences for both groups ($p<0.0001$) for PPD measurements. However, no statistical difference was observed between two groups (inter group comparison) at any of the time points. (Table 3)

Table 3: Probing pocket depth (PPD) and Relative attachment level (RAL)

Groups		PPD baseline	PPD 3months	PPD 6months	PPD 9months	p-value (time)	RAL baseline	RAL 3months	RAL 6months	RAL 9months	p-value (time)
OFD	N	20	20	20	12	0.000[c]*	20	20	20	12	0.000[d*]
	Mean	6.6000	4.9000	3.9000	3.0833		12.1000	11.1000	9.9500	9.0000	
	SD	1.14248	1.02084	0.91191	0.51493		1.25237	1.44732	1.27630	1.12815	
PRGF	N	20	20	20	12	0.000[c]*	20	20	20	12	0.000[d*]
	Mean	6.5500	4.4500	3.6500	3.2500		12.6000	10.9500	9.8000	8.7500	
	SD	1.05006	0.68633	0.81273	0.62158		1.50088	1.31689	1.36111	1.42223	
p-value		0.886[b]	0.149[a]	0.572[a]	0.444[a]		0.260[b]	0.734[b]	0.721[b]	0.638[b]	

a: Mann-Whitney test
b: student test
c: Freidman test
d: ANOVA for repeated measures
*(upto 6 months or upto 9 months)
# upto 6 months
$ upto 9 months

4.2.2 Relative attachment level (RAL):

Intra-group comparison within all time points showed statistically high significant differences for both groups ($p<0.0001$) for RAL measurements. However, no statistical difference was observed between two groups (inter group comparison) at any of the time points. (Table 3)

4.2.3 Gingival marginal level (GML):

It was seen that the GML was found significantly improving from baseline to 9 months in both groups ($p<0.01$), but not at 6 months for OFD group ($p=0.092$). When compared between both groups, significant favorable improvement was seen in PRGF treated group at all time points, suggesting continuous vertical creeping of the free gingival margin in the PRGF group at 3, 6 and 9 months. (Table 4)

Table 4: Gingival marginal level (GML)

Groups		GML baseline	GML 3months	GML 6months	GML 9months	p-value (time)
OFD	N	20	20	20	12	0.092[c$]
	Mean	1.9000	1.6000	1.4000	1.5000	0.010[c#]
	SD	0.71818	0.59824	0.88258	1.00000	
PRGF	N	20	20	20	12	0.000[c*]
	Mean	1.9000	1.0500	0.8500	0.6154	
	SD	0.78807	0.68633	0.74516	0.65044	
p-value		0.977 [a]	0.010 [a]	0.041 [a]	0.022 [a]	

a: Mann-Whitney test
b: student test
c: Freidman test
# upto 6 months
$ upto 9 months
GML- Gingival marginal level

4.2.4 Change in PPD,RAL and GML

The change in PPD at 3, 6 and 9 months from baseline, showed no significant difference between both groups. However, the change in RAL at 3 and 6 months showed favorable improvement in PRGF treated group (p=0.003 at 3 months & p=0.05 at 6 months). The change in GML was also significantly better in PRGF treated group, showing statistically significant improvement at all time points. (Table 5)

This suggests the gingival recession was significantly reducing in the PRGF group at 3, 6 and 9 months and the clinical attachment gain was significantly higher in the PRGF at 3 and 6 months.

Table 5: Change in PPD, RAL and GML at all time points

Groups		ΔPPD 3months	ΔPPD 6months	ΔPPD 9months	p-value (time)	ΔRAL 3months	ΔRAL 6months	ΔRAL 9months	p-value (time)	ΔGML 3months	ΔGML 6months	ΔGML 9months	p-value (time)
OFD	N	20	20	12	0.000[c] 0.000[d]	20	20	12	0.000[c] 0.000[d]	20	20	12	0.301[c] 0.157[d]
	Mean	1.7000	2.7000	3.6667		1.0000	2.1500	3.2500		0.3000	0.5000	0.4167	
	SD	0.73270	0.80131	1.07309		0.79472	1.03999	0.96531		0.7124	0.76089	0.79296	
PRGF	N	20	20	12	0.000[c] 0.003[d]	20	20	12	0.000[c] 0.000[d]	20	20	13	0.022[c] 0.046[d]
	Mean	2.1000	2.9000	3.5000		1.6500	2.8000	3.6667		0.8500	1.0500	1.3077	
	SD	0.91191	1.37267	1.24316		0.58714	0.76777	.98473		0.48936	0.39403	0.48038	
P-value		0.162[a]	0.862[a]	0.729[b]		0.003[a]	0.05[a]	0.342[a]		0.003[a]	0.004[a]	0.003[a]	

a: Mann-Whitney test
b: student test
c: Freidman test
d: Wilcoxon test
*(upto 6 months or upto 9 months)
# upto 6 months
$ upto 9 months

4.2.5 Clinical Attachment Loss (CAL)

The clinical attachment loss was significantly lower in the PRGF group at 3 and 6 months (p=0.002 at 3 months, p=0.043 at 6 months), However, the clinical attachment gain was significantly higher in the PRGF group at 3 months (p=0.005) and borderline significance at 6 months (p=0.067). (Table 6)

Table 6: Clinical attachment loss at all time points

Groups		CAL baseline	CAL 3months	CAL 6months	CAL 9months	p-value (time)	ΔCAL 3months	ΔCAL 6months	ΔCAL 9months	p-value (time)
OFD	N	20	20	20	12	0.000[b*]	20	20	12	0.001[b]
	Mean	8.5000	6.5000	5.3000	4.5833		2.0000	3.2000	4.0833	0.000[c]
	SD	1.10024	1.00000	1.21828	1.24011		0.85840	1.15166	1.44338	
PRGF	N	20	20	20	12	0.000[b*]	20	20	12	0.000[b]
	Mean	8.4500	5.5000	4.5000	3.9167		2.9500	3.9500	4.8333	0.000[c]
	SD	1.31689	0.94591	1.19208	0.79296		1.14593	1.35627	1.33712	
p-value		0.897[a]	0.002[a]	0.043[a]	0.131[a]		0.005 [a]	0.067 [a]	0.200 [a]	

a: student test
b: ANOVA for repeated measures
c: student test for repeated measures
*(upto 6 months or upto 9 months)

4.3 Radiographic measurements

4.3.1 Intra-bony defect depth (IBDD):

IBDD was recorded at baseline, 6 months and 9 months. The change in IBDD at both 6 months (p=0.020) was found to be significantly improved for PRGF treated group. (Table 7) The linear radiographic bone gain was significantly higher in the PRGF treated group at 6 months. Border line significance was observed at 9 months for PRGF group (p=0.081)

4.3.2 Intra-bony defect area (IBDA):

At 6 months, IBDA was found to be significantly low for PRGF treated group than OFD group (p=0.013). (Table 7) However, the IBDA found at 6 and 9 months was significantly improved in both groups (p=0.001) than baseline. (Table 8)

4.3.3 Intra-bony defect area fill percent (%IBDAF):

No significant difference was observed in %IBDAF for both groups at the end of 6 and 9 months. (Table 7)

Table7: Inter-group comparison of radiological parameters at baseline, 6 and 9 months

	Treatment	N	Mean	SD	p-value
IBDD_baseline (mm)	OFD	20	4.2460	0.77152	0.610[a]
	PRGF	20	4.3890	0.97455	
IBDA-baseline (mm^2)	OFD	20	5.5585	1.37429	0.056 [a]
	PRGF	20	6.8995	2.55778	
IBDD_6months (mm)	OFD	20	3.5460	0.85729	0.513 [a]
	PRGF	20	3.3650	0.87719	
IBDA_6months (mm^2)	OFD	20	4.3515	1.19962	0.013 [a]
	PRGF	20	5.5745	1.73150	
IBDAF_6months (mm^2)	OFD	20	1.1675	0.59048	0.655[b]
	PRGF	20	1.3310	0.96037	
%IBDAF _6months	OFD	20	22.1340	7.94764	0.117 [a]
	PRGF	20	18.4600	6.46233	
IBDD_9months (mm)	OFD	12	3.0458	0.82774	0.928 [a]
	PRGF	12	3.0158	0.78308	
IBDA_9months (mm^2)	OFD	12	4.0208	1.23301	0.040 [a]

	PRGF	12	5.3650	1.74068	
IBDAF _9months (mm^2)	OFD	12	1.6258	0.97961	0.021[b]
	PRGF	12	2.2258	1.44923	
%IBDAF_9months	OFD	12	28.9617	11.25353	0.827[a]
	PRGF	12	28.1233	6.77296	
ΔIBDD_6months (mm)	OFD	20	0.7000	0.40786	0.020[a]
	PRGF	20	1.0240	0.43363	
ΔIBDD_9months (mm)	OFD	12	1.2225	0.45947	0.081[a]
	PRGF	12	1.5658	0.46119	

a: student test
b: Mann-Whitney test
IBDD- Intra-bony defect depth
IBDA- Intra-bony defect area
IBDAF – Intra-bony defect area fill

Table 8: Intra-group comparison of change in radiological parameters from baseline upto 6 and 9 months

		OFD p-value (time)	PRGF p-value (time)
IBDD	Upto 6months	0.000[a]	0.000[a]
	Upto 9 months	0.000[c]	0.000[c]
IBDA	Upto 6months	0.000[a]	0.000[b]
	Upto 9 months	0.000[c]	0.000[d]
IBDAF		0.002[b]	0.002[b]
%IBDAF		0.002[b]	0.000[a]
ΔIBDD		0.001[a]	0.009[a]

a: Student test for repeated measures
b: Wilcoxon test
c: ANOVA for repeated measures
d: Friedman test

4.4 Clinical Success Criteria:

The success criteria were assessed based on CAL change by 1.5 mm and linear radiographic bone gain of 1.0 mm.

At 6 months, the PRGF was significantly superior to the OFD (50% Vs 15%) in the number of sites that achieved CAL change by 1.5 mm and linear radiographic bone gain of 1.0 mm. (Table 9)

At 9 months, the PRGF was border-line significance than the OFD (83% Vs 50%) in the number of sites that achieved CAL change by 1.5 mm and linear radiographic bone gain of 1.0 mm. (Table 10)

Table 9: Clinical success at 6 months

6 months		Group		Total
		OFD	PRGF	
Success* (Cochrane et al. 2016)	,00	17	10	27
	1,00	3	10	13
Total		20	20	40
p-value		0.018[a]		

a: χ^2 test
*Success criteria: CAL change by 1.5 mm and linear radiographic bone gain of 1.0 mm

Table 10: Clinical success at 9 months

9 months		Group		Total
		OFD	PRGF	
Success* (Cochrane et al. 2016)	,00	6	2	8
	1,00	6	10	16
Total		12	12	24
p-value		0.083[a]		

a: χ^2 test
*Success criteria: CAL change by 1.5 mm and linear radiographic bone gain of 1.0 mm

4.5 Patient reported outcomes:

4.5.1 Swelling in surgically treated area

Table 11. Swelling

		d1	d2	d3	d4	d5	d6	d7	p-value (time)
OFD	N	20	20	20	20	20	20	20	
	Mean	,7000	,6000	,4500	,3000	,2000	,0500	,0500	0.000[b]
	SD	,97872	,88258	,82558	,57124	,41039	,22361	,22361	
PRGF	N	20	20	20	20	20	20	20	
	Mean	,7000	,7500	,7000	,4500	,4000	,1000	,0500	0.000[b]
	SD	,86450	,91047	,97872	,75915	,68056	,30779	,22361	
p-value		0.905[a]	0.595[a]	0.416[a]	0.616[a]	0.390[a]	0.553[a]	1.000[a]	

a: Mann-Whitney test
b: Friedman test

4.5.2. Bleeding in surgically treated area

Table 12. Bleeding

		d1	d2	d3	d4	d5	d6	d7	p-value (time)
OFD	N	20	20	20	20	20	20	20	
	Mean	,5000	,3500	,0000	,0000	,0000	,0000	,0000	0.000[b]
	SD	,94591	,81273	,00000	,00000	,00000	,00000	,00000	
PRGF	N	20	20	20	20	20	20	20	
	Mean	,3500	,2500	,1000	,0500	,0000	,0000	,0000	0.004[b]
	SD	,74516	,63867	,30779	,22361	,00000	,00000	,00000	
p-value		0.521[a]	0.683[a]	0.152[a]	0.317[a]	1.000[a]	1.000[a]	1.000[a]	

a: Mann-Whitney test
b: Friedman test

4.5.3 Level of pain in surgically treated area

Table 13. Level of pain

		d1	d2	d3	d4	d5	d6	d7	p-value (time)
OFD	N	20	20	20	20	20	20	20	
	Mean	22,0000	20,0000	16,5000	12,5000	8,0000	7,0000	5,2500	0.000[b]
	SD	29,30780	25,54665	29,24938	26,33289	18,23819	15,59352	10,93943	
PRGF	N	20	20	20	20	20	20	20	
	Mean	7,5000	6,5000	4,0000	3,0000	1,0000	,2500	,0000	0.000[b]
	SD	14,82352	12,68028	12,31174	9,23381	3,07794	1,11803	,00000	
p-value		0.132[a]	0.117[a]	0.036[a]	0.112[a]	0.177[a]	0.064[a]	0.019[a]	

a: Mann-Whitney test
b: Friedman test

CHAPTER 5. DISCUSSION

This double blind, split-mouth, randomized controlled trial recruited twenty patients presenting two contra-lateral sites each, treated either with PRGF along with OFD and IMP, or OFD and IMP alone. All the patients completed their 6 months follow-up. However, only 12 patients could complete their 9 months follow-up. There was a loss to follow-up for 8 patients at the end of 9 months due to the lockdown restrictions for present COVID 19 pandemic.

There are many advantages of using PRGF than other platelet concentrates in treatment of periodontal defects. First, the specificity of this technique in which the buffy coat layer (between the blood cell base and acellular plasma) are not harvested in order to avoid collection of leukocytes. Since buffy coat layer contains most leukocytes, the final PRGF was a plasma suspension with almost no leukocytes which improves the homogeneity of the product, and thus prevents the destruction of surrounding cells at the healing sites by the reactive oxygen species and MMPs produced by the neutrophils (88)**.**

Second, addition of calcium chloride calcium chloride promotes the for formation of native thrombin, mimicking the physiological process and enabling a more sustained release of growth factors, which might be crucial to proper tissue repair and wound healing. Moreover, this procedure provokes immunological reactions and the risk of the disease transmission associated with the use of exogenous bovine thrombin (89).

Due to above mentioned advantages, the present study was conducted to assess the additive effect of PRGF used along with OFD and IMP. To best of our knowledge, till date, there are no published data on use of PRGF with OFD and IMP in treatment of intra-bony defects in chronic periodontitis patients.

The clinical parameters like PPD, RAL, SSPI, SSGI were improved for both groups at the end of post-operative follow-up at 3, 6 and 9 months. However, no statistical significant difference was observed between both groups at the end of any follow-up period. Interestingly, When compared between both groups, significant favorable improvement was seen in GML for PRGF treated group at all time points, suggesting continuous vertical creeping of the free gingival margin in the PRGF group

at 3, 6 and 9 months. This could be due to the favorable effect of PRGF on fibroblast and epithelial cell proliferation. Many literatures evaluated the proliferative effect of PRGF on keratinocytes and fibroblasts, that can explain our results showing improvement in GML. Andreas Bayer et al in 2017 investigated the effect of PRGF on keratin 1 and keratin 10 gene expression. Microscopic analysis of PRGF treated keratinocytes revealed that those keratinocytes went terminal differentiation. Also, they concluded that this particular feature is advantageous in healing chronic wounds (90). Anitua et al in 2016 performed an in-vitro study on dermal fibroblasts were isolated from healthy volunteers. For PRGF preparation, blood samples were collected from two different age groups (18–35 years and 50+ years) and in three different concentrations (5%, 10% and 20%) and the effects of PRGF on cell proliferation was evaluated. It was seen in that study that, cell proliferation was enhanced along with the increment of PRGF dose concentration after 72 hours. The addition of PRGF leads to increased proliferation of dermal fibroblasts ($p<0.01$). The result was maximum with 20% PRGF treatment which shown 2.3 times increased cell proliferation. In case of cell migration, it shows a dose-dependent increment (5%, 10% and 20%) when compared with control ($p<0.01$). Finally, it showed 8 fold increment in cell migration (91). Vahabi et al in 2015 performed an in vitro study and concluded that, at the time interval of 24, 48 and 72 hours, PRGF treatment caused statistically significant proliferation of fibroblast when compared to control (123%±2.25%, 102%±2.8% and 101%±3.92%, respectively). At 24 hours interval PRGF membrane had significant effect compared to negative control. And fibroblastic proliferation was higher in PRGF group (92).

The favorable result in reduction of SSBOP in PRGF treated group could be attributed to its anti-inflammatory and regenerative effects. PRGF exerts more potent regenerative and anti-inflammatory effects than autologous serum on ocular surface fibroblasts treated with pro-inflammatory IL-1β and TNFα (91). In an in-vitro study, the effects of PRGF preparations on cell proliferation were determined using human periosteal cells. In the PRGF preparations, both red blood cells and WBCs were almost completely eliminated, and platelets were concentrated by 2.84-fold. The absence of leukocytes could be a reason for not exerting any further pro-inflammatory effects in the area of regeneration (93).

The linear radiographic bone gain was significantly higher in the PRGF treated group at 6 months. Border line significance was observed at 9 months for PRGF group. This gain can be attributed to the positive response of PRGF on osteoblast by reducing oxidative stress and increasing VEGF expression and thereby showing cytoprotective effects on them. Oxidative stress can cause production of reactive oxygen species which can further leads to reduction in wound healing. These enzymes are controlled by antioxidant response element (ARE) and (erythroid-derived 2)-like2 (Nrf2) plays a critical part in activation of ARE-driven genes. Currently, it has been shown that PRGF can help in up regulation of VEGF which in turn helps in decrease the damage caused by the oxidative pathway. M Tohidnezhad et al in 2014 did an in-vitro study and concluded that an increment in VEGF expression in osteoblast in PRGF treatment group. Activation of Nrf2/ARE system in osteoblast via PRGF promotes regeneration of bone. And PRGF has shown cytoprotective effect on osteoblast (94).

The reduction in level of pain in surgically treated area was found to significantly higher for PRGF treated group. This is the first of its kind assessing the level of pain postoperatively after periodontal surgery treated with PRGF. In a previous study by Haraji et al 2012 assessed the reduction of level of pain in sockets with alveolar osteitis treated with PRGF (95).

Few randomized controlled trials has been performed to evaluate the regenerative effects of PRGF applied alone or in combination to other biomaterials for treatment of periodontal intra-bony defects. Shabnam Khalifehzadeh et al in 2018 performed a clinical trial with 8 patients with debridement, 1% metformin, PRGF in two walled intrabony defects. Both the test and control groups showed significant improvements in the clinical parameters, in terms of radiographic changes remarkable improvements were also seen and concluded the favourable response of PRGF in improving all clinical parameters (96). Maryam Bojarpour et al in 2018 performed regenerative therapy in 3 walled intrabony defects using PRGF. The test group was treated with xenogenic collagen membrane and PRGF while, in the control only the membrane was applied after the debridement. A statistically significant difference was found in radiographic indices among the groups post-surgery (P=0.009).

They concluded that the use of PRGF improved all the clinical and radiological parameters except gingival index (97). Ravi et al in 2017 performed a randomized control trial to evaluate the effectiveness of PRGF+GTR versus GTR alone in the chronic periodontitis patients having 2 or 3 walled bony defects and did follow up for 6 months. The CAL change was statistically significant in both the groups, control group (5.42 ± 1.99) and the test group (5.99 ± 1.77). Mean radiographic bone fill was 1.06 ± 0.81 and 1.0 ± 0.97 in the control group and test group, respectively. However, they concluded that as there was not much of a significant difference between both the groups, so there was no significant additive effect of PRGF along with GTR in terms of both clinical and radiological parameters. A comparative clinical trial done by Sadatmansouri et al in 2010 examined the effect of PRGF alone and PRGF with bovine porous bone material with GTR in cases of intrabony defects. At 6 months follow up both the groups showed significant reduction of P.I, G.I and CAL. The mean PPD decrease in PRGF group was 4.1 ± 1.52 mm and 4.5 ± 1.5 mm on buccal and lingual sites, respectively ($P < 0.0001$); and 3.6 ± 0.9 mm in the test group. They have concluded that PRGF alone can provide almost similar results like PRGF +bovine bone material +GTR membrane (98).

PRGF also exerted its beneficial effect in improving the clinical parameters when used as intra-pocket application in shallow periodontal pockets treated non-surgically. Panda et al in 2020 performed a randomized controlled trial to check the effectiveness of intra-pocket application of PRGF along with SRP. 22 patients (44 sites) were randomly allocated for the study and evaluated for months to check for the clinical parameters. There was a statistically significant difference in both the groups while the results favouring the PRGF+SRP group in terms of PPD ($p = 0.007$) and RAL ($p = 0.021$) at the end of 6 month follow-up. The authors concluded that a single intra-pocket administration of PRGF after non-surgical periodontal therapy was effective in clinical attachment gain and reduction of CAL (99).

The treatment success reported in various studies may differ which is likely to be in part to the varying morphology of initial defects. In this study, some defects were 3 wall defects, while others were a combination of 1-2 walls. It has been reported that 2 and 3 wall defects have the highest potential for regeneration when grafting procedures are used (100). In addition, the defects varied

according to depth and width, with the aforementioned study reporting better predictability with deep, narrow defects versus shallow, wide ones. It is hard to control these variables in a clinical investigation, but the potential effects of this variability on the results need to be realized. However, even in the best circumstances, it is impossible to find matching osseous defects in test and control groups. However, randomization employed in the present study may help to control this variability.

Limitations

The study was not able to assess the 9 months follow-up measurements due to the effect of COVID-19 pandemic. It would have been interesting to assess the radiological parameters using advanced modalities of assessment like cone beam computerized tomography (CBCT). The study included smokers, and also had defect variation in the contra-lateral sites. It was impossible to standardize the defect morphology to assess the exact bone fill and bone gain.

CHAPTER 6. CONCLUSION

- PRGF when used in adjunct to OFD and IMP was found to improve the gingival marginal level and reduce the bleeding on probing, thereby reducing the inflammation and promoting vertical creeping of gingival margin over a period of 6 months.
- It was also found to have greater linear radiographic bone gain with PRGF treated group compared to that observed in OFD and IMP group.
- PRGF treated group was also found to show higher and statistically significant clinical success rate. PRGF was significantly superior to the OFD (50% Vs 15%) in the number of sites that achieved clinical attachment gain by 1.5 mm and linear radiographic bone gain of 1.0 mm at the end of 6 mpnths. At 9 months, the PRGF was border-line significance than the OFD (83% Vs 50%) in the number of sites that achieved CAG by 1.5 mm and linear radiographic bone gain of 1.0 mm.
- PRGF treated group was also found to show significant reduction in level of pain experienced by patients at the end of day 3 and day 6 compared to OFD and IMP group.
- Overall, PRGF was found to beneficial in terms of improved clinical attachment gain and radiographic linear bone gain, when used in adjunct to OFD and IMP for management of periodontal intra-osseous defects.

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