T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PERIODONTOLOGY

CLINICAL AND RADIOGRAPHIC COMPARISON OF PLATELET RICH FIBRIN COMBINED WITH BOVINE DERIVED XENOGRAFT VERSUS BOVINE DERIVED XENOGRAFT ALONE IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS

PhD THESIS

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İSTANBUL - 2016

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Periodontology Doctorate Programme

Title of the Thesis : Clinical and Radiographic Comparison of Platelet Rich Fibrin Combined with Bovine Derived Xenografts versus Bovine Derived Xenograft Alone in the Treatment of Periodontal Intrabony Defects

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Examination Date : 22.01.2016

This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

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APPROVAL

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DECLERATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor and programme director **Prof. Dr. Recep Selçuk Yılmaz** for his guidance and mentorship through my PhD education and for sharing his vast knowledge and expertise.

I am thankful to **Prof. Dr. Bahar Eren Kuru** for conveying her knowledge with exceptional skill in teaching at every opportunity through the programme.

I would like to thank **Prof. Dr. Ülkü Noyan** for sharing her clinical expertise and advises with great sincerity.

I am very grateful to **Assoc. Prof. Dr. Gökser Çakar** for her sincere guidance and advices through my undergraduate and post graduate education as well as for her enormous assistance and involvement in the research.

I would like to thank **Assoc. Prof. Dr. Şebnem Dirikan İpçi**, **Assoc. Prof. Dr. Hare Gürsoy**, **Dr. Ebru Özkan Karaca**, **Dr. Ogül Leman Tunar**, **and Dr. Can Yenigün** for sharing their expertise.

I would like to thank **Begüm Atalay, D.D.S.** for her extraordinary support, **Dr. Ö. Samed Kuka**, **Dr. Gizem İnce** for their uplifting friendship**,** and my colleagues from the department, **Dr. Sarah Alsulimani Büyükdağ**, **Dr. Sadberg Cihangir Hamud and Dr. Dudu Otçuoğlu** for their help and creating a heartwarming atmosphere through the PhD programme.

I am especially grateful to **Dr. Cem Ünlüçerçi** for being a great friend enlightening me with his advises through my education.

I would like to thank, my mother and father, **S. Harika Düzenli** and **Recep Düzenli** for their tremendous support, guidance and encouragement in every step I take with their endless love, trust and wisdom.

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LIST of SYMBOLS and ABBREVIATIONS

BDX	Bovine derived xenograft
BMP	Bone morphogenic protein
DFDBA	Demineralized freeze dried bone allograft
DSD	Deepest site of the defect
EMD	Enamel Matrix Derivative
GTR	Guided tissue regeneration
HA	Hydroxyapatite
IBDD	Intrabony defect depth
IGF-I	Insulin like growth factor-I
IGF-II	Insulin like growth factor-II
MSTL	Marginal soft tissue level
OFD	Open flap debridement
PD	Probing depth
PDGF	Platelet derived growth factor
PGF	Polypeptide growth factor
PI	Plaque Index
PRF	Platelet rich fibrin
PRP	Platelet rich plasma
RAL	Relative attachment level
Rad BG	Radiographic bone gain
Rad BL	Radiographic bone level
RBL	Relative bone level
SBI	Sulcus bleeding index
SD	Standard deviation
SRP	Scaling and root planing
TGF-β	Transforming growth factor-beta
®	Registered Trademark

ABSTRACT

Düzenli, D. (2016). Clinical and Radiographic Comparison of Platelet Rich Fibrin Combined with Bovine Derived Xenograft versus Bovine Derived Xenograft Alone in the Treatment of Periodontal Intrabony Defects. Yeditepe University, Institute of Health Sciences, Department of Periodontology, PhD Thesis, Istanbul.

The aim of the present study was to compare the clinical and radiographic effects of platelet rich fibrin (PRF) combined with bovine derived xenograft (BDX) to the use of BDX alone in the treatment of periodontal intrabony defects of advanced chronic periodontitis patients. Twenty advanced chronic periodontitis patients with a mean age of 45.60 ± 10.01 were enrolled in the present study. A total of 92 periodontal intrabony defects with an associated probing depth (PD) of ≥ 5 mm and an intrabony component of ≥ 3 mm were treated with either PRF + BDX or BDX alone. At baseline and 12 months after surgery, plaque and sulcus bleeding indices, PD, marginal soft tissue level, relative attachment level, relative bone level together with radiographic bone level were recorded. Uneventful healing was observed in all cases. At 12 months, both treatment groups revealed significant clinical and radiographic improvements when compared to baseline ($p < 0.01$). Regarding the deepest site of the defects, following changes in clinical and radiographic parameters were observed at 12 months after surgery for PRF + BDX and BDX groups respectively; a mean PD reduction of 3.35 ± 1.25 (3) mm and 3.0 ± 1.41 (3) mm, attachment gain of 3.02 ± 1.26 (3) mm and 2.15 ± 1.35 (2) mm, gingival recession of 0.33 ± 0.52 (0) mm and 0.79 ± 0.56 (1) mm, clinical bone gain of 2.74 ± 1 (3) mm and 2.10 ± 1.2 (2) mm and radiographic bone gain of 2.63 ± 0.98 (3) mm and 2.24 ± 1.03 (2) mm. Intergroup analysis demonstrated significant attachment gain, gingival recession, clinical bone gain ($p < 0.01$) and radiographic bone gain ($p < 0.05$) in favor of the PRF + BDX group. Results obtained in this study revealed that the use of both PRF + BDX combination and BDX alone yields significant improvements in clinical and radiographic parameters compared to baseline and the use of PRF + BDX combination yields better results in regards to attachment gain, clinical and radiographic bone gains.

Key Words: Platelet Rich Fibrin, Bovine Derived Xenograft, Intrabony Defect, Periodontal Regeneration, Advanced Chronic Periodontitis

ABSTRACT (TURKISH)

Düzenli, D. (2016). Periodontal Kemik İçi Defektlerin Tedavisinde Trombositten Zengin Fibrin ve Sığır Kaynaklı Kemik Grefti Kombinasyonu ile Tek Başına Sığır Kaynaklı Kemik Grefti Uygulamalarının Klinik ve Radyografik Olarak Karşılaştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Periodontoloji ABD., Doktora Tezi. İstanbul.

Bu çalışmada ileri kronik periodontitis teşhisi konmuş hastalarda bulunan periodontal kemik içi defektlerin tedavisinde trombositten zengin fibrin (TZF) ve sığır kaynaklı kemik grefti (SKKG) kombinasyonu ile tek başına uygulanan SKKG'nin klinik ve radyografik olarak karşılaştırılması amaçlandı. Çalışmamıza yaş ortalaması 45.60 ± 10.01 olan 20 kronik periodontitis hastası dahil edildi. Başlangıç tedavisini takiben sondalama derinliği (SD) ≥ 5 mm ve kemik içi defect derinliği ≥ 3 mm olan 92 defekt TZF + SKKG kombinasyonu veya sadece SKKG uygulanarak tedavi edildi. Operasyondan önce ve 12 ay sonra, plak ve dişeti olugu kanama indeksleri, rölatif dişeti kenarı konum seviyesi, SD, rölatif ataşman ve kemik seviyeleri ile radyografik kemik seviyesi ölçümleri yapıldı. Tüm vakalarda iyileşme sorunsuz gerçekleşti. Operasyon sonrası 12. ayda her iki tedavi grubunda da klinik ve radyografik parametrelerde başlangıca göre anlamlı iyileşme tespit edildi ($p < 0.01$). Defektin en derin noktası göz önüne alındığında, TZF +SKKG ve SKKG gruplarında sırasıyla 3.35 ± 1.25 (3) mm ve 3.0 ± 1.41 (3) mm SD azalması, 3.02 ± 1.26 (3) mm ve 2.15 ± 1.35 (2) mm ataşman kazancı, 0.33 ± 0.52 (0) mm ve 0.79 ± 0.56 (1) mm dişeti çekilmesi ile 2.74 ± 1 (3) mm ve 2.10 ± 1.2 (2) mm klinik ve 2.63 ± 0.98 (3) mm ve 2.24 ± 1.03 (2) mm radyografik kemik kazancı saptandı. Gruplar arasında yapılan değerlendirmelerde, ataşman kazancı, dişeti çekilmesi, klinik kemik kazancı ($p < 0.01$) ve radyografik kemik kazancı ($p < 0.05$) parametrelerinde TZF + SKKG lehine anlamlı fark tespit edildi. Bu çalışmadan elde edilen bulgular, hem TZF + SKKG kombinasyonunun, hem de tek başına SKKG uygulamalarının, ileri kronik periodontitisli hastalarda gözlenen periodontal kemik içi defektlerde başlangıca göre anlamlı klinik ve radyografik iyileşme sağladığını ve TZF + SKKG uygulamasının ataşman kazancı, klinik ve radyografik kemik kazancı açışından ilave katkısının bulunduğunu göstermektedir.

Anahtar Kelimeler: Trombositten Zengin Fibrin, Sığır Kaynaklı Kemik Grefti, Kemik İçi Defekt, İleri Kronik Periodontitis, Periodontal Rejenerasyon

1. AIM and INTRODUCTION

Chronic periodontitis is an inflammatory disease of polymicrobial origin and is clinically characterized by apical migration of sulcus epithelium along the root surface resulting in clinical attachment loss, deep pockets and alveolar bone loss (1). The treatment strategy is aimed at disease prevention, arresting disease progression, regenerating lost periodontal tissues and maintaining the achieved treatment outcomes (2, 3).

Ultimate goal of periodontal treatment is to recreate a functional epithelial seal at the most coronal portion of the tissues, a new connective tissue attachment inserted into the previously exposed root surface to form the periodontal ligament and the dentogingival apparatus, a new acellular extrinsic fiber cementum and new alveolar bone. Thereby, creating functionally oriented periodontal ligament attachment to previously diseased root cementum with new cementum and bone formation leading to a complete regeneration of both soft and hard tissues (4).

In modern periodontology, along with flap operations, bone grafts, guided tissue regeneration (GTR) technique and biologic mediators such as enamel matrix derivatives (EMD), bone morphogenic proteins (BMP), polypeptide growth factors (PGF), platelet rich plasma (PRP), platelet rich fibrin (PRF) and their combinations are being used.

Periodontal regeneration consists of numerous biologic mechanisms including but not limited to cell migration/adhesion/proliferation and differentiation (5). PGF's are accepted as local and systemic proteins which regulate these mechanisms. Therefore, they present a potential use in periodontal regenerative treatments (5).

Platelets exhibit a vital role in wound healing and are a natural resource for PGF's (6). During the aggregation process, α-granules of platelets release PGF's to the wound site (5). Extensively studied growth factors to date are platelet derived growth factor (PDGF), insulin-like growth factor I and II (IGF-I, IGF-II) and transforming growth factor β (TGF-β). In vitro studies demonstrated that rate of cell proliferation and differentiation was increased and animal studies showed an improvement of periodontal regeneration with the use of PDGF's and IGF's (7, 8). Studies investigating periodontal regeneration on humans with the use of these PGF's are limited and this subject is still a topic of interest (9-12).

One natural source for PGF's is PRF. It is a second generation autologous platelet concentrate with a simple and quick preparation protocol (13). PRF, with its three-dimensional resilient fibrin matrix and ability to slowly release PGF's and matrix

glycoproteins for more than 7 days, is regarded as a promising biomaterial for improving periodontal wound healing and regeneration (14).

Recently, the clinical effects of PRF are being studied on sinus lift procedures, alveolar crest augmentations, preservation of extraction sockets, root coverage procedures, and treatment of periodontal defects (15-32). In the literature, comparison of PRF use to open flap debridement (OFD) in the treatment of periodontal intrabony defects revealed clinical and radiographic improvements on the outcomes in favor of PRF (26, 29-31). A comparative evaluation of PRF with PRP and EMD was carried out in two different studies for the treatment of periodontal intrabony defects (27, 28). Results of PRF were found to be similar to PRP. However, authors suggested the use of PRF over PRP based on ease of use and simple preparation protocol. The comparison with EMD revealed similar results except for defect resolution percentage. When the efficacy of PRF was compared to demineralized freeze dried bone allograft (DFDBA) and autogenous bone grafts, results were found to be similar and differences were statistically insignificant (24, 25). Therefore it was suggested that PRF could be an alternative to bone graft materials. On the other hand, Lekovic et al. (23) demonstrated that using PRF + bovine derived xenograft (BDX) combination significantly improves the treatment outcomes over PRF alone. When the additional effect of PRF in combination with various bone grafts was compared to bone grafts alone, it was shown that PRF significantly improves the probing depth (PD) reduction and attachment gain (21, 22).

It is proposed that PRF, as an autologous biomaterial, improves the clinical and radiographic outcomes of periodontal regenerative treatments and its success is enhanced by the use of grafting materials. However, current literature does not contain any studies evaluating the additional effect of PRF when combined with BDX. Therefore, aim of the present study was to compare the clinical and radiographic effects of PRF + BDX to the use of BDX alone in the treatment of periodontal intrabony defects.

2. BACKGROUND INFORMATION

2.1. Regenerative Periodontal Therapy

Periodontitis is a complex interaction between an infection and a susceptible host which is associated with alveolar bone loss and is diagnosed by increase in PD, loss of clinical attachment and radiographic evidence of bone loss (33).

Objectives of periodontal treatment are to achieve; elimination of the infection caused by the pathogen microorganisms, an increase in periodontal attachment, decrease in periodontal pocket depths and, no or a minimal increase in gingival recession. Ultimate goal is to recreate/regenerate the periodontal tissues to a pre-disease state. These objectives in turn leads to a functional and healthy use of the natural dentition as a whole (34, 35).

In the 1970s and 1980s, pocket elimination was the main objective of periodontal therapy, and resective techniques such as gingivectomy or apically positioned flap procedures were commonly performed to eliminate the periodontal pocket and allow access to the root surface for debridement (36). The goal was to eliminate the infection, and together with resective osseous surgeries, natural architecture of the bone and gingival tissues were aimed to be recreated apical to their natural position (37). Resective approaches may be applied with great success, however they are far from regenerating the original structure of the periodontium. Due to apical repositioning of soft and hard tissues, esthetic problems may arise and in the case of poor oral hygiene, residual pocket formation may be observed (34). Following resective periodontal treatments, histological studies showed that healing is completed by the formation of a long junctional epithelium rather than a new connective tissue attachment between the alveolar bone and root cementum due to faster proliferation and differentiation of epithelial cells compared to mesenchymal cells (38, 39).

Successful periodontal treatment depends on the re-formation of all periodontal tissues; an epithelial seal, deposition of new acellular extrinsic fiber cementum and insertion of functionally oriented Sharpey's fibers into the root surface, and restoration of alveolar bone (40). Thus, generating a similar form and function found in the intact, native periodontal attachment is called periodontal regeneration (4, 41-44). To reach this treatment goal, various treatment approaches were developed.

Chronologic development of these approaches are listed respectively:

- Use of bone graft materials (autogenous grafts, allografts, xenografts, alloplastic grafts),
- Use of barrier membranes (GTR technique),
- Use of biologic mediators (EMD, BMP, PRP, PRF),
- Combined use of the above-mentioned approaches.

The first approach was the application of bone graft materials such as autogenous bone obtained from the patients, allografts obtained from human donors, xenografts obtained from different species and fabricated alloplastic graft materials. The studies conducted on the use of these graft materials showed limited attachment gain together with limited radiographic bone fill. Histologic studies revealed healing by long junctional epithelium rather than true regeneration (4, 34, 38, 45-49). A defect fill of 60-65% was observed however, presence of residual defects led the researchers to develop more advanced approaches (42).

In 1976, Melcher (50) proposed the idea that following periodontal surgical treatment, the outcome of the healing process is defined by the first cell population occupying the wound site (cells from; epithelium, connective tissue, alveolar bone or periodontal ligament). In 1982, Nyman et al. (39) stated that regeneration can only occur when cells originating from the periodontal ligament occupied the wound site and the epithelial and connective tissue cells must be isolated from the area. The concept was then adapted into the clinical practice setting by the use of non-resorbable and resorbable barrier biomaterials. For this purpose, during the periodontal surgery, placement of physical barriers to prevent apical migration of the epithelium and gingival connective tissue cells and to provide an isolated space for the migration of periodontal ligament cells and mesenchymal cell on the exposed root surface, was proposed to promote periodontal regeneration. This is the biologic basis of the GTR technique (40).

There are vast amount of studies showing successful use of graft materials and barrier membranes alone or in combination for the treatment of periodontal intrabony defects (42, 51, 52). Despite the success of GTR technique, technical sensitivity of the procedure, difficulty in primary closure of the flaps, high risk of membrane exposure and microbial contamination are the shortcomings of this approach (38, 42, 53). These shortcomings led the researchers to develop alternative strategies and materials to enhance the regenerative potential of the treatments. One of the latest approaches is the use of biologic mediators.

They are used to enhance the innate potential of regeneration and compensate for the shortcomings of more conventional approaches (35).

Tissue engineering is a biomedical science and research field which specializes in building new functional tissues to replace the damaged or lost ones. Building a new tissue from ground up requires an extracellular matrix or scaffold together with appropriate and regular signals and sufficient progenitor cells. All these components should be supported with adequate blood supply (54).

Success in tissue engineering relies on 3 main factors, progenitor cells to populate, appropriate matrix or scaffold to support and biologic signal molecules to regulate cell differentiation all of which serve to create a functional tissue. The interaction of these 3 factors defines the qualitative and quantitative properties of the newly formed tissue (4, 54).

During/after periodontal surgery, a cascade of innate healing events defines the outcome of the treatment. This may either lead to regeneration or repair. These events may be basically summarized as; cell migration and proliferation, angiogenesis, extracellular matrix formation and remodelling. These cellular events in turn result in 3 main phases of wound healing; hemostasis, granulation tissue formation and tissue remodelling (55).

Immediately after surgical trauma, vasoconstriction limits the bleeding thus allows for fibrin clot formation. This fibrin clot acts as a matrix for cell migration and proliferation for the cells of periodontal ligament, alveolar bone and gingiva. Following the closure of the surgical wound, fibrin clot fills the debrided defect space and adheres to the surrounding tissues and seals the wound site. As the blood is outside the vessel, platelet activation occurs and starts to release an arsenal of mediators such as adhesive proteins, thrombospondin, fibronectin, fibrinogen, transforming growth factor-α, TGF-β and variety of PDGF's. These mediators regulates the proliferation and migration of osteoblasts, fibroblasts, smooth muscle cells, leucocytes, monocytes and neutrophils together with cells of the periodontal supporting structures. Therefore, the undisturbed formation of the fibrin clot as matrix for cells to migrate/proliferate on exhibits a great importance on how the periodontal wound healing or regeneration will take place (56).

In the search of complete periodontal regeneration, principles of tissue engineering are being used to promote cementogenesis, osteogenesis and periodontal ligament formation. In this regard, bone grafts for their osteogenic, osteoinductive and osteoconductive properties (41, 48, 57, 58), GTR to promote specific cell types (51, 52,

59-64) and biologic mediators to manipulate cell to cell interactions are being used (65-75).

A recent research subject on periodontal tissue engineering is the use of PDGF. PDGF has been shown to increase attachment gain and defect fill when used in periodontal intrabony defects. Platelet rich plasma started to be widely used as a platelet concentrate, however in recent years a second generation platelet concentrate PRF has become topic of interest.

2.2. Bone Grafts and Their Mechanism of Action in Regenerative Periodontal Therapy

Graft materials used in periodontal regenerative therapies are expected to act on the defect site by means of osteogenesis, osteoinduction and osteoconduction. Osteogenesis is the process of new bone formation in which live cells inside the graft material takes part in the formation of new bone tissue. Live osteoblasts of the endosteum and bone marrow stem cells found in the graft are capable of promoting new bone formation when placed in soft tissues and activates faster bone formation in hard tissues (57, 76). Osteoinduction is the process of stimulation of osteoprogenitor cells to differentiate into osteoblasts which then take part in new bone formation. Thus, osteoinductive graft materials can be used to promote bone regeneration. Osteoconduction is a physical property of the graft material, it creates a scaffold which allows for osteoblasts and mesenchymal cells to attach to the grafted site. Therefore, osteoconductive materials help new bone formation by facilitating bone apposition from the surrounding bone tissue. Unlike the osteogenetic grafts, when placed inside the soft tissues, osteoconductive materials does not promote new bone formation. Therefore, osteoconductive graft materials need the presence of live bone and mesenchymal cells in the grafted area to work (77).

The ideal bone graft used in periodontal regenerative treatments should have the following properties:

- Non-antigenic,
- Not toxic or carcinogenic,
- Osteoinductive and osteoconductive,
- Easy to manipulate,
- Does not cause ankylosis or root resorption,
- Easily obtained in adequate amounts,

- Low cost,
- Stable and physically resilient.

2.3. Xenografts

Xenografts are produced from two main sources; bovine and coral. With different fabrication techniques, grafts produced from these sources exhibit biocompatible and human bone like structural properties. When compared to synthetic grafts, xenografts show a closer structural similarity to the human bone (57).

Bovine derived xenografts are produced by impregnating the bone with ethylene diamine for 24 hours to separate the organic components therefore, harvesting the natural bone minerals (57). Harvested inorganic calcium matrix is then sterilized and deemed ready for use. This sterilized inorganic material is devoid of organic components and is a corticocancelleus hydroxyapatite (HA) skeleton with micro/macroporous structure. The natural HA structure acts as a source of calcium for new bone formation and during the remodeling phase retains its physical size (78).

Bovine derived xenografts exhibit osteoconductive and partially osteoinductive properties (79, 80). With its porous structure and higher mineral content compared to human bone, BDX provide an osteoconductive scaffold and integrate to grafted site to a higher extend (57). Due to their scaffolding abilities, these grafts are widely used for alveolar ridge reconstructions and sinus augmentations (81, 82).

When xenografts are used alone, osteogenic cells from the defect margins starts new bone formation towards the graft material. However, when used in combination with autogenous bone grafts to enhance the regenerative potential, new bone formation may start within the graft where osteogenic cells are present (83). Histologic investigations revealed that there is no fibrous tissue formation or spaces between the bone and the HA structure (57).

Xenografts are resorbed by osteoclasts over time (57), however some studies showed that this resorption process is extremely slow. In a clinical study conducted by Schlegel and Donath (84), mandibular bone defects were filled with 100 % BDX and showed that even after 6 years, graft material persisted in the defect sites. Sartori et al. (85) examined a patient following maxillary sinus augmentation with BDX for 10 years. Resorption rate was found to be 3.55 % per month up to 2 years and for the following 8 years, resorption rate was gradually decreased to 0.58 % (85). It can be concluded that, in contrast to other graft materials, xenografts allow for new bone formation around the graft without completely being resorbed.

The effects of xenografts in the treatment of periodontal intrabony defects have been investigated since early 1980's. In an animal study conducted by Sonis et al. (86), the role of BDX in artificially created intrabony defects in dogs were investigated. Defects were divided into 2 groups; BDX and OFD. Clinical and histological assessments were done at 1, 3, 6 and 12 months after the procedure. It was shown that the graft was well tolerated by the tissues and no inflammatory reactions were present. Histologic assessment of the BDX revealed that new attachment could be observed at 1 month and graft was replaced with new bone at 3 months. No histological differences could be observed between the groups at 6 and 12 months. At 12 months, PD reduction was 1.22 mm and 0.81 mm for BDX and control groups, respectively. Researchers concluded that BDX was easy to use and could promote new bone formation, therefore could be used effectively in the treatment of periodontal intrabony defects.

In the literature, numerous clinical studies evaluated the use of BDX in the treatment of periodontal intrabony defects.

Gupta et al. (87) investigated the clinical effects of BDX (test group) compared to OFD (control group) in the treatment of 30 patients with a total of 40 intrabony defects presenting PD ≥ 6 mm and intrabony component of ≥ 3 mm. Probing depth reduction, attachment gain and radiographic bone gain were evaluated. Probing depth reduction in test and control groups were 2.80 mm and 4.05 mm; 1.75 mm and 2.65 mm at 3 and 6 months, respectively. Attachment gain in test and control groups were 2.80 mm and 4.00 mm; 1.75 mm and 2.60 mm at 3 and 6 months, respectively. Radiographic bone gain in test and control groups were 2.02 mm and 3.27 mm; 0.82 mm and 1.17 mm at 3 and 6 months, respectively. Defect fill in test and control groups were 37.1 % and 56.5 %; 20.5 % and 28.6 % at 3 and 6 months, respectively. The authors concluded that the use of BDX compared to OFD presented significantly higher PD reduction, attachment gain and radiographic bone fill. Therefore, BDX could be used as a valid option in treatment of periodontal intrabony defects.

Richardson et al. (48) compared the clinical effects of BDX and DFDBA in the treatment of 17 patients with 22 intrabony defects presenting PD ≥ 5 mm and intrabony component ≥ 3 mm. Probing depth reduction, attachment gain and bone fill were evaluated and re-entry was performed at 6 months. Probing depth reduction was 3.00 mm and 2.00 mm, attachment gain was 3.60 mm and 2.60 mm, defect fill was 3.00 mm (56 %) and 2.40 mm (47 %) for BDX and DFDBA groups, respectively. Even though PD reduction, attachment gain and defect fill values were found to be higher in the BDX

group, differences were found to be statistically insignificant between the groups. The authors concluded that both materials could be used with success in the treatment of periodontal intrabony defects.

Scabbia and Trombelli (58) evaluated the clinical outcomes of BDX in comparison to synthetic HA in the treatment of 24 patients with 24 intrabony defects presenting PD ≥ 6 mm and intrabony component ≥ 4 mm. At 12 months, BDX group exhibited PD reduction of 4.40 mm, attachment gain of 4.00 mm and radiographic bone gain of 3.10 mm. Same parameters for HA group were 4.20 mm, 2.90 mm and 2.50 mm, respectively. Statistical analysis revealed no significant differences between the groups. According to the results, it was suggested that both materials could be used for the treatment of intrabony defects.

Camelo et al. (59) treated 4 teeth with poor prognosis in advanced chronic periodontitis patients. Two defects with initial PD of 9 mm and 10 mm were treated with BDX and other two with PD of 10 mm and 11 mm were treated with a combination of BDX and collagen membrane. Clinical, radiographic and histological results were compared. Clinical and radiographic measurements were repeated after 6 and 9 months. Treated teeth were extracted as a block with the surrounding bone for histological investigation. Probing depth reduction of BDX treated teeth were 4.00 mm and 6.00 mm, and 8.00 mm and 5.00 mm for the combination group. Attachment gain for BDX treated teeth were 4.00 mm and 5.00 mm, whereas 7.00 mm and 4.00 mm for the combination group. Histological analysis of both groups revealed new cementum, new periodontal ligament and new bone formation. According to the histological data, BDX treated teeth exhibited 5.1 mm and 5.2 mm of new cementum and 4.2 mm and 4.8 mm of new bone formation. The combination group exhibited 7 mm and 7.6 mm of new cementum, 4.5 mm and 5.3mm of new bone formation. It was shown that BDX was able to create new cementum and bone, and the results could be improved with the addition of collagen membranes.

A similar study by Nevins et al. (63) also revealed new bone and cement formation when defects were treated with BDX and with BDX + collagen membrane. On the other hand, a study by Hartman et al. (61) concluded that BDX alone had a significant impact on regeneration whereas addition of collagen membrane had no additive effect on the outcome of the treatment.

Numerous studies on the use of BDX for the treatment of periodontal intrabony defects revealed that BDX may be used with clinical and radiographic success and

promotes periodontal regeneration in the treatment of periodontal intrabony defects (48, 58, 59, 61, 63, 86-88).

In recent years, autologous platelet concentrates have become a great interest in periodontal regenerative medicine. There are limited number of studies examining the added benefit of PRP when used together with BDX in periodontal intrabony defects.

Hanna et al. (68) evaluated the clinical effects of PRP + BDX in comparison to BDX alone. Study included 13 patients with 26 defects, presenting PD ≥ 6 mm and intrabony component ≥ 4 mm. Patients were either treated with PRP + BDX (test group) or BDX (control group) alone. At 6 months, test and control groups showed a PD reduction of 3.54 mm and 2.53 mm, attachment gain of 3.15 mm and 2.31 mm, respectively. Differences in terms of PD reduction and attachment gain between the groups were found to be statistically significant in favor of the test group. Authors concluded that the use of PRP together with BDX presented improved results compared to the use of BDX alone in the treatment of intrabony defects.

A similar study was conducted by Ouyang et al (89). 10 patients with 17 intrabony defects with PD ≥ 6 mm and intrabony component ≥ 4 mm were treated. Nine defects were treated with PRP + BDX (test group) and the remaining 8 were treated with BDX (control group) alone. At 12 months, test and control groups resulted in PD reduction of 4.78 mm and 3.48 mm, attachment gain of 4.52 mm and 2.85 mm, defect fill of 4.56 mm (73 %) and 2.88 mm (47 %), respectively. Results for the test group were found to be better in all clinical parameters and differences were found to be statistically significant. Authors concluded that combined use of PRP and BDX achieved better results compared to the use of BDX alone.

Döri et al. (90) conducted a study on 30 patients where 15 defects were treated with PRP + BDX (test group) and 15 defects were treated with BDX alone (control group). At 12 months, following results were obtained for test and control groups respectively; PD reduction of 5.2 mm and 5.3 mm, attachment gain of 4.6 mm and 4.7 mm. The authors concluded that both PRP + BDX and BDX alone were effective in regards to PD reduction and attachment gain. However, there was no added benefit in using PRP together with BDX when compared to the use of BDX alone in the treatment of periodontal intrabony defects.

Recently, regarding the above-mentioned results of PRP, the new generation of autologous platelet concentrate, PRF, has become the focus of researchers to improve the regenerative treatment outcomes.

2.4. Platelet Rich Fibrin

Platelet rich fibrin is a second generation platelet concentrate developed by Choukroun et al. (91) for the clinical use in oral and maxillofacial surgery. It has several advantages with regards to other platelet concentrates, with simplified preparation, ease of application and cost effectiveness. In contrast to the first generation PRP preparations, PRF does not require the use of anticoagulants or bovine thrombin. Preparation only requires a table top centrifuge and blood collection kit. It can be regarded simply as centrifuged blood. Since anticoagulants are not used, platelets in the blood sample that contact the collection tube starts the coagulation process in few minutes. Initially, fibrinogen is located mainly in the upper part of the collection tube. During the centrifugation process, thrombin in the blood sample turns the fibrinogen in to fibrin. Centrifugation concentrates the fibrin clot in the middle part of the tube, upper part being acellular plasma and lower part being red corpuscles. Critical aspect in the preparation process is the quick handling of the blood samples, this prevents diffuse polymerization of the fibrin clot which in turn leads to inconsistent PRF formation (92).

2.5. Rationale and Biologic Principles of Periodontal Regeneration With The Use of Platelet Rich Fibrin

The use of a platelet concentrates such as PRF in surgical procedures is a method for accelerating wound healing and tissue maturation. Structure of the PRF consists of three dimensional fibrin matrix which is slowly polymerized in a trimoleculer or equilateral structure unlike the first generation PRP which constitutes of bilateral or tetramolecular fibrin branch junctions formed by sudden polymerization which are unfavorable for cytokine enmeshment and cellular migration. PRF presents with mainly equilateral junctions. This type of fibrin mesh allows for a flexible fibrin network that is able to support cytokine entrapment and cellular migration thus results in flexible, elastic and resilient PRF membrane. These properties are comparable to a natural fibrin matrix therefore explains the cicatrical capacity of this biomaterial (92).

This structure allows the platelets, leucocytes, PGF's and circulating stem cells to be incorporated into the PRF biomaterial. Platelets are formed from the megakaryocytes in the bone marrow. These discoidal and anuclear cells have a life span of 8-10 days. When activated, these cells secrete the contents stored in the α-granules inside the cytoplasm. The activation causes the cytokines to be released, which promotes cell migration and proliferation that are crucial in the healing process. Among the PGF's, TGF-β is a platelet cytokine which is a strong fibrosis agent and promotes the synthesis of matrix molecules

such as collagen I and fibronectin. Therefore, TGF-β is an inflammatory regulator with a capacity to induce fibrous cicatrization. PDGF's are essential for mesenchymal cell migration, proliferation and survival. IGF-I/II are cell multiplication mediators responsible for proliferation and differentiation for most cell types. In addition to these cytokines, the PRF matrix also contains glycosaminoglycans such as heparin and hyaluronic acid which have a strong affinity with circulating peptides and presents strong capacity to promote cell migration and healing process. Therefore, PRF can be considered as a healing biomaterial (93). Due to the specific fibrin polymerization and three-dimensional structure of the PRF membrane, above-mentioned cytokines and matrix glycoproteins can be slowly released in significant amounts for at least 7 days (13, 14, 94).

The slow polymerization process of the PRF biomaterial is proposed to promote leucocyte degranulation. This degranulation results in an increased secretion of interleukins that are of leucocytic origin and are trapped in the fibrin network during the polymerization process and are slowly released. The study conducted by Dohan et al. (95) revealed that interleukin-1 beta, interleukin-6, tumor necrosis factor-alfa and interleukin-4 are found in greater quantities inside the PRF exudate compared to measurements obtained from plasma and sera samples. With a high content of immune cytokines, authors concluded that PRF could be considered as an immune node capable of defending against infections due to chemotactic and neovascularization capacities of these cytokines.

In conjunction with the biologic features, in vitro studies revealed that PRF promotes the proliferation of different types of cells including osteoblasts (96), gingival fibroblasts as well as periodontal ligament fibroblasts (97).

The above-mentioned properties of the PRF biomaterial forms the rationale for using this material as a biologic membrane in regenerative periodontal treatment of intrabony defects. Using the PRF membrane is proposed to cover and protect the blood clot and/or graft material inside the defect area and also promotes the soft tissue healing which results in periosteal coverage of the defect site together with a resilient gingiva. This promoted periosteum coverage functions as a natural barrier between the soft tissue and hard tissue compartments, therefore acts as a regenerative barrier thereby enhancing the regenerative outcomes (98).

2.6. Studies on Platelet Rich Fibrin and Combinations

Promising biological features of PRF led the researchers to investigate the in vitro and in vivo effects of this second generation autologous platelet concentrate. Animal studies conducted on tibial defects in pigs, sinus augmentations in dogs, calvarial defects in rabbits with histologic analysis revealed positive results in regards to hard tissue healing and promotion of new bone formation (99-102). Based on these results, the clinical effects of PRF in the treatment of periodontal intrabony defects has become a topic of interest.

Pradeep et al. (28) compared the effects of OFD, PRF + OFD and PRP + OFD for the treatment of 3-wall intrabony defects in 54 chronic periodontitis patients with PD ≥ 5 mm and intrabony component ≥ 3 mm. Clinical and radiographic parameters were recorded at baseline and 9 months. Both PRF and PRP groups exhibited better results compared to OFD in regards to PD reduction (3.77 mm and 3.77 mm vs 2.97 mm) and attachment gain (3.17 mm and 2.93 mm vs 2.83 mm) as well as defect fill (55.41 % and 56.85 % vs 1.56 %). Differences in PD reduction and defect fill were found to be statistically significant when PRF and PRP groups were compared to OFD. Differences were found to be statistically insignificant between PRF and PRP groups. The authors concluded that PRF being less technique sensitive, easier and cheaper to prepare and less time consuming, it might be a better treatment option compared to PRP.

A controlled clinical trial conducted by Thorat et al. (31) investigated the effect of PRF in the treatment of intrabony defects. 32 patients and 32 defects with a PD ≥ 3 mm and intrabony component ≥ 3 mm were treated. At 9 months, following results were obtained for the PRF and OFD groups respectively; PD reduction of 4.56 mm and 3.56 mm, attachment gain of 3.69 mm and 2.13 mm, defect fill of 46.92 % and 28.66 %. Both groups showed statistically significant improvements compared to baseline. Intergroup comparison revealed superior results in favor of PRF group.

Sharma and Pradeep (30) conducted a randomized controlled clinical trial to compare the clinical and radiographic effects OFD (control group) vs PRF (test group) in the treatment of 3-wall intrabony defects. 42 patients with 56 3-wall intrabony defects with PD ≥ 5 mm and intrabony component ≥ 3 mm were treated. Clinical and radiographic measurements were taken at baseline and 9 months after surgery. Statistical analysis revealed significantly greater results for the test group compared to control in terms of PD reduction (4.55 mm vs 3.21 mm), attachment gain (3.31 mm vs 2.77 mm) and defect fill (48.26 % vs 1.80 %). The authors concluded that PRF was effective in the treatment of 3-wall intrabony defects.

Joseph et al. (29) investigated the clinical outcomes of PRF for the treatment of intrabony periodontal defects. A split-mouth design study was conducted on15 patients with contralateral defects. Defects with PD ≥ 6 mm and intrabony component ≥ 3 mm were treated. Test group was treated with PRF + OFD and control group was treated with OFD alone. At 12 months, both groups showed significant improvements compared to baseline. Statistical analysis revealed significant improvements for the test group compared to control for PD reduction (4.67 mm vs 2.40 mm), attachment gain (4.73 mm vs 1.40 mm) and defect fill (1.93 mm vs 0.64 mm). Within the limits of the study, it has been stated that the use of PRF significantly improves both clinical and radiographic parameters as well as reduces post-operative pain and discomfort. The authors also underlined the need for multicenter studies with larger sample sizes and longer follow up periods.

Ajwani et al. (26) treated 20 patients presenting 40 intrabony defects with PD ≥ 5 mm and intrabony component ≥ 3 mm. Defects were treated with either PRF or OFD. At 9 months, both treatment groups resulted in improved hard and soft tissue parameters compared to baseline except for gingival recession. Data evaluation revealed PD reduction of 1.90 mm and1.60 mm, attachment gain of 1.80 mm and 1.30 mm for PRF and OFD groups, respectively. Differences were found to be statistically insignificant. Only defect fill was found to be statistically significant in favor of the PRF group (1.45 mm vs 0.80 mm). The authors stated that the use of PRF can improve the defect fill compared to OFD and emphasized the selection of PRF use with its simple preparation protocol and low cost.

Using a split mouth design, Lekovic et al. (23) investigated the effects of BDX when combined with PRF in treatment of periodontal intrabony defects. 17 paired intrabony defects were treated randomly with either PRF or PRF + BDX combination. Re-entry was performed 6 months after surgery. At 6 months, PRF + BDX group presented significantly improved results for buccal/lingual sites compared to PRF alone group in terms of PD reduction (4.47 mm/4.29 mm vs 3.35 mm/3.24 mm), attachment gain (3.82 mm/3.72 mm vs 2.24 mm/2.12 mm) and defect fill (4.06 mm/3.94 mm vs 2.21 mm/2.06 mm). The authors suggested that the additional use of BDX improved the PD reduction, attachment gain and defect fill compared to treatment with PRF alone.

Shah et al. (25) treated 20 patients presenting 40 bilateral intrabony defects with PD ≥ 5 mm in a split mouth design. Defects were treated with either PRF or DFDBA. At 6 months, statistical analysis revealed no significant differences between the PRF and

DFBDA groups in terms of PD reduction (3.67 mm vs 3.70 mm), attachment gain (2.97 mm vs 2.97 mm) and gingival recession (0.43 mm vs 0.72 mm). Both groups showed clinical improvements compared to baseline. The authors stated that both DFDBA and PRF showed similar results and can be used to treat periodontal intrabony defects.

Mathur et al. (24) evaluated the treatment outcomes of PRF (test group) and autogenous bone graft (control group) in periodontal intrabony defects. A total of 38 defects in 25 patients with PD ≥ 5 mm and radiographic defect depth ≥ 3 mm were treated. At 6 months, both groups presented improved clinical results compared to baseline. However, intergroup comparison for test and control groups revealed no statistically significant differences in terms of PD reduction (2.67 mm and 2.4 mm), attachment gain (2.53 mm and 2.67 mm) and defect fill (2.93 mm and 2.66 mm), respectively. The authors pointed out that both treatment modalities were suitable in the treatment of intrabony defects.

Gupta et al. (27) studied the effects of PRF compared to EMD in the treatment of periodontal intrabony defects of 32 patients with 44 intrabony defects. Three wall defects with PD ≥ 5 mm and intrabony component ≥ 3 mm were treated. Clinical measurements and cone beam computerized tomography images were taken both at baseline and at 6 months. All examined parameters showed statistically significant improvements over baseline values. Intergroup comparison revealed significantly better results for defect resolution in favor of EMD group (43.07 % vs 32.41 %). Differences on other parameters were found to be statistically insignificant. The authors reported that both EMD and PRF were effective in treating intrabony defects with EMD being more successful in terms of defect resolution. The authors suggested that multicenter studies with large sample sizes and long follow-up periods should be conducted on the use PRF.

Elgendy et al. (22) conducted a study comparing the effects of nanocrystalline HA (control group) and PRF + nanocrystalline HA (test group) on 20 patients presenting bilateral defects with PD ≥ 6 mm in the treatment of periodontal intrabony defects. At 6 months, following data were obtained for the control and the test groups respectively; PD reduction of 3.30 mm and 3.33 mm, attachment gain of 3.50 mm and 3.55 mm. The difference at 6 months were found to be statistically significant compared to baseline for both groups. However, intergroup analysis revealed more successful results for PD reduction and attachment gain in favor of the test group. The authors concluded that, the clinical outcomes of intrabony defects may be improved with the additional use of PRF membrane compared to use of nanocrystalline HA alone.

In a study conducted by Bansal and Bharti (21), 10 patients with almost identical intrabony defects on both sides of the jaw with PD ≥ 6 mm were randomly treated with DFDBA (control group) or PRF + DFDBA combination (test group). Both groups showed statistically significant improvements compared to baseline in terms of PD reduction and attachment gain. At 6 months, following changes were observed for control and test groups respectively; PD reduction of 3.1 mm and 4.0 mm, attachment gain of 2.3 mm and 3.4 mm. Differences between the groups were found to be statistically significant in favor of the test group. The authors concluded that PRF + DFDBA combination yields better results compared to the use of DFDBA alone in the treatment of periodontal intrabony defects.

Agarwal et al. (20) conducted a split-mouth study on 30 patients with 60 bilateral intrabony defects presenting PD ≥ 6 mm and intrabony component ≥ 4 mm. Patients were either treated with PRF + DFDBA (test) or DFDBA alone (control). In both groups, grafted defects were covered with a PRF membrane. At 12 months, both groups resulted in significant improvements compared to baseline. Comparing test and control groups, differences in PD reduction (4.15 mm and 3.60 mm), attachment gain (3.73 mm and 2.61 mm) and defect fill (3.50 mm and 2.49 mm) were statistically significant in favor of the test group. The authors concluded that the use of PRF + DFDBA mixture combination is more effective compared to the use of DFDBA alone in the treatment of intrabony defects.

These limited number of studies investigating the regenerative treatment of periodontal intrabony defects with PRF and various combinations revealed improved treatment outcomes. However, current literature lacks the information on effects of PRF when used in addition to BDX. Therefore, the purpose of this study is to compare the clinical and radiographic effects of PRF + BDX to the use of BDX alone in the treatment of periodontal intrabony defects.

3. MATERIALS and METHODS

3.1. Patient and Defect Selection

Following inclusion criteria were used for the selection of the patients and the defect sites:

1. Systemically healthy individuals,
2. No history of periodontal treatment,
3. No medication used for past 6 months which may affect the periodontal tissues,
4. No history of allergic reactions to the medications and materials to be used in the study,
5. Socio-economic eligibility,
6. Non-smoker,
7. Radiographic findings of vertical bone loss,
8. Plaque index (PI) < 1 following initial periodontal therapy,
9. PD ≥ 5 mm following initial periodontal therapy,
10. At least 2 mm of keratinized tissue at the facial aspect of the defect related site,
11. Presence of 3+2+1-, 3+2-, 3+1-, 3-, 2+1- and 2-wall intrabony defects with an intrabony component ≥ 3 mm.

3.2. Initial Periodontal Therapy

Before starting the periodontal treatments, all patients were educated about periodontal diseases, microbial dental biofilm as the cause of the disease and methods for preventing biofilm accumulation (103). Patients were instructed to use modified Bass method for brushing, dental floss and/or interdental brush as oral hygiene appliances (104). Instructions were given on demonstration models and followed by hands on application.

Each patient received full mouth supra/subgingival scaling and root planing (SRP) under local anesthesia using ultrasonic scalers[1] and Gracey curettes[2] as part of the initial periodontal therapy. Following SRP, polishing was done with polishing pastes using brushes and plastic cones attached to a low speed rotary hand piece. Each session, patients were evaluated in regards to their oral hygiene practices. Corrections were made and additional instructions were given when necessary. Occlusal adjustment was performed if trauma from occlusion was diagnosed. Trauma from occlusion was evaluated by examining the obvious presence of fremitus in centric and protrusive movements. All

[1] **Piezon® OEM built-in kit**, EMS, Nyon, Switzerland.
[2] **Gracey,** SG, Hu-Friedy, Chicago, USA.

caries and endodontic problems were eliminated by endodontics and restorative departments.

Patients were re-evaluated for their eligibility according to the selection criteria 3 months after the completion of initial periodontal therapy. Patients who were successful to achieve necessary oral hygiene level, able to comply with the treatment and recall schedules were advanced to the surgical treatment phase.

3.3. Research Groups and Plan

A group of patients who applied to Yeditepe University Faculty of Dentistry Department of Periodontology with complaints of gingival bleeding, tooth mobility, tooth migration, and based on clinical and radiographic examinations diagnosed as advanced chronic periodontitis were included in the present study (105). After an explanation of all aspects of the study, as well as the alternative treatment regimens, an informed consent to participate in the study was obtained from the patients. From this population, a total of 20 patients, 9 male and 11 female, whose ages ranged from 30 to 63 were randomly assigned to two groups. Randomization was carried out using coin toss method. First coin toss was done to define the groups as head or tail. Following coin tosses were performed to allocate the patients to the treatment groups. First group was treated with PRF + BDX (10 patients) and the second group was treated with BDX alone (10 patients). Research plan is presented in Figure 1. According to this plan, patients were re-evaluated 3 months after initial periodontal therapy. For each patient, individual occlusal stents were made for both upper and lower jaws. Standard radiographies together with intraoral photographs were taken and clinical measurements were recorded before the surgery. Surgical treatment of each patient was done in two sessions, one for the lower and one for the upper jaw. During the surgery, measurements related to the intrabony defect components were recorded together with intraoral photographs. Following the surgery, patients were recalled for control and professional tooth cleaning/polishing at 1 week. Sutures were removed at 2 weeks. During the 12-month follow-up period, patients were recalled for once a week for the first 2 months, once every 2 weeks up to 3 months and once a month until the end of the 12-month observation period. At 12 months, all clinical measurements were repeated and radiographies were taken.

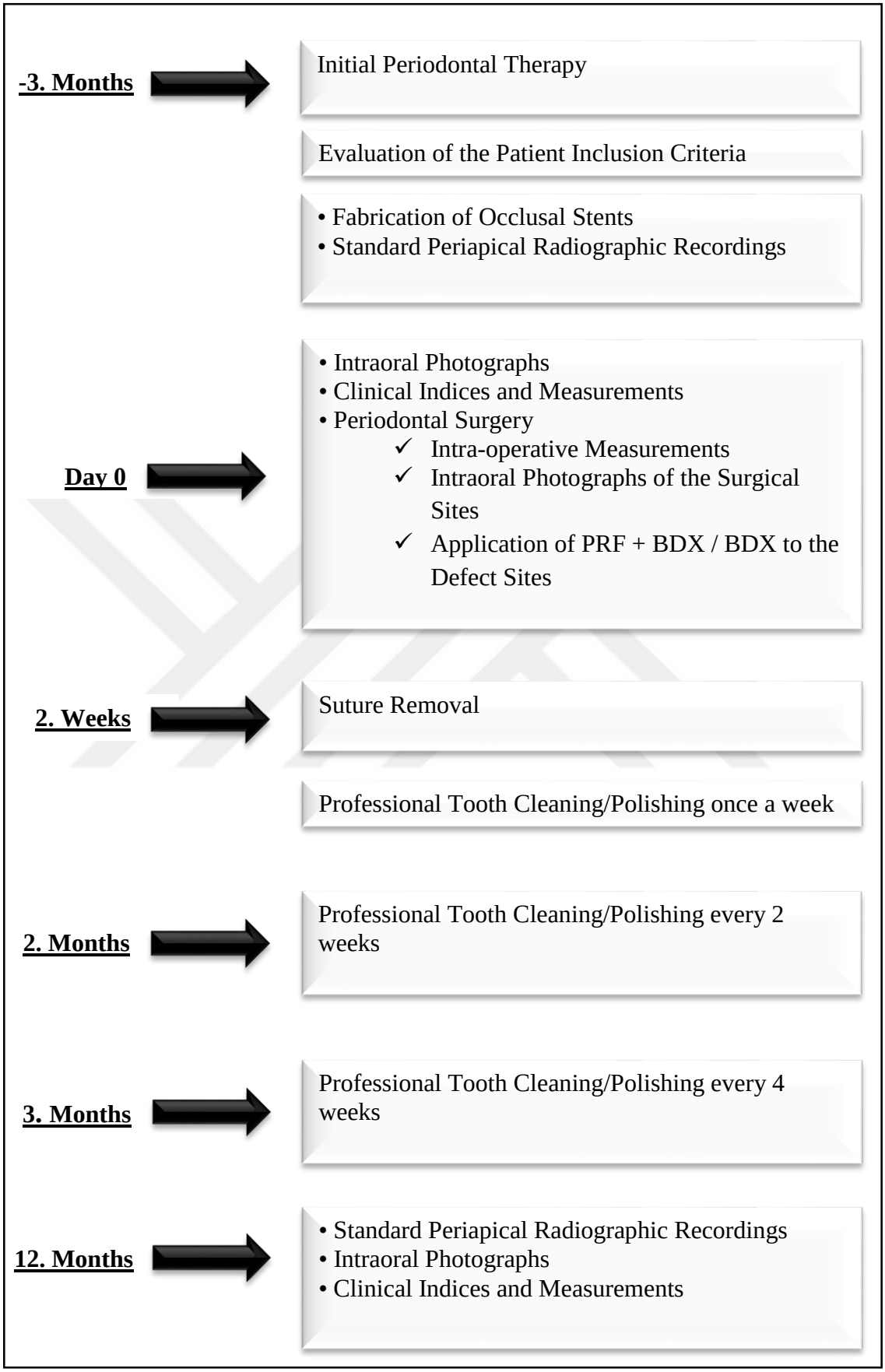


Figure 1. Research plan.

3.4. Clinical Indices and Measurements

Intra-examiner calibration was evaluated on 5 patients, measuring at least 6 teeth twice within a 48 hour time frame. The examiner was regarded as calibrated when initial and 48 hour measurements were at ≥ 90 % consistency to the mm (106). Recordings were done in an order to prevent any negative interference between different clinical measurements. All measurements were recorded to the specially designed data sheets, before, during and 12 months after surgeries (Figures 2-4).

An explorer and a periodontal probe[3] with 15 mm length and 0.4 mm diameter were used for the measurements. Occlusal acrylic stents were fabricated to standardize measurements and decrease the possibility of error with regards to the probe positioning and angulation. Stents were made to sit on the occlusal surfaces covering 1/3 of the crown area from the buccal and lingual sides of the treated teeth. Six grooves were placed on the buccal and lingual sides for mesial, distal and mid-coronal positions on the acrylic stents to obtain identical probe positioning and angulation before and after the treatment. The indices and the measurements used in this research are summarized below:

3.4.1. Plaque Index

Teeth were isolated by cotton rolls and dried using air syringe. The microbial dental biofilm on the teeth surfaces were evaluated by an explorer at four points; mesio-buccal, mid-buccal, disto-buccal and mid-lingual. Scores between 0-3 were given for each point to detect PI (107) (Figure 2).

3.4.2. Sulcus Bleeding Index

The clinical condition of the gingival tissues was evaluated using Sulcus Bleeding Index (SBI). Evaluation was done for both buccal and lingual sides, at 6 points including mesial, distal and mid-coronal aspects. Each point was probed carefully without traumatizing the tissues with a periodontal probe being held parallel to the long axis of the teeth and moved from mesial and distal line angles to the col region of the interdental papillae. Index values were recorded with the scores of 0 to 5 according to both the clinical appearance of the gingiva and the bleeding after probe movement (108) (Figure 2).

3.4.3. Marginal Soft Tissue Level

Marginal soft tissue level (MSTL) was measured using the individual occlusal stents. Mesial, distal and mid-coronal aspects were measured using the 6 grooves on the stent

[3] **PCP 15 UNC**, Hu-Friedy, Chicago, USA.

for both buccal and lingual surfaces. Distance between the apical edge of the stent and the gingival margin was measured using a periodontal probe (Figures 2, 5).

3.4.4. Probing Depth

Periodontal probe was placed into the periodontal pocket with the aid of the grooves on the individual occlusal stents. Mesial, distal and mid-coronal sites were measured using the 6 grooves on the stents for both buccal and lingual surfaces from bottom of the periodontal pocket to the gingival margin (Figures 2, 5).

3.4.5. Relative Attachment Level

Periodontal probe was placed into the periodontal pocket with the aid of the grooves on the individual occlusal stents. Mesial, distal and mid-coronal aspects were measured using the 6 grooves on the stent for both buccal and lingual surfaces. Distance from bottom of the periodontal pocket to the apical edge of the stent was recorded as relative attachment level (RAL) (Figures 3, 5).

3.4.6. Relative Bone Level

Following local anesthesia, periodontal probe was placed into the periodontal pocket with the aid of the grooves on the individual occlusal stents. Probe was advanced apically until the tip of the probe was in contact with the alveolar bone. Mesial, distal and mid-coronal aspects were measured using the 6 grooves on the stent for both buccal and lingual surfaces. Distance from the alveolar bone to the apical edge of the stent was recorded as relative bone level (RBL) (Figures 3, 5).

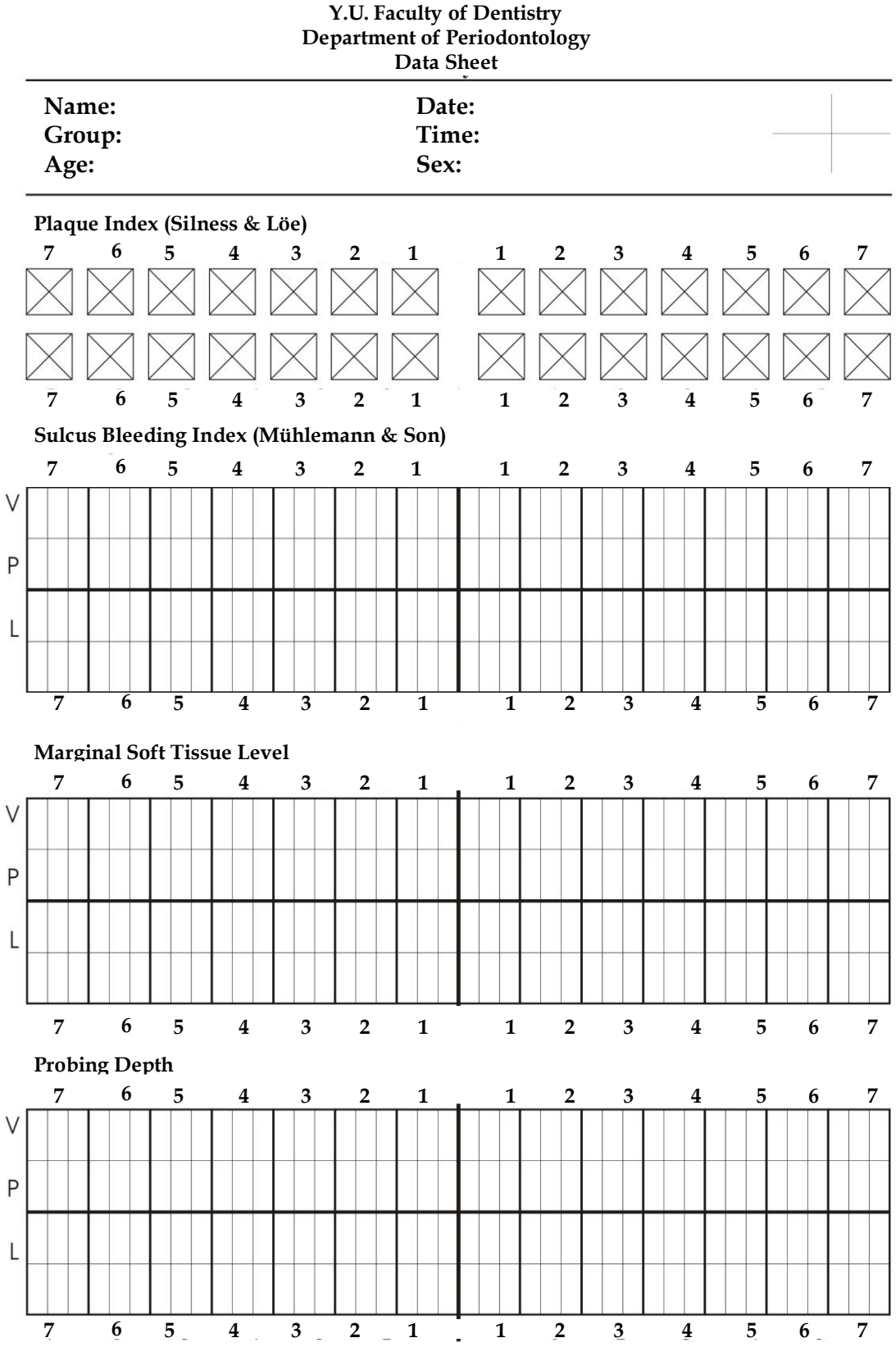


Figure 2. Clinical indices and measurements.

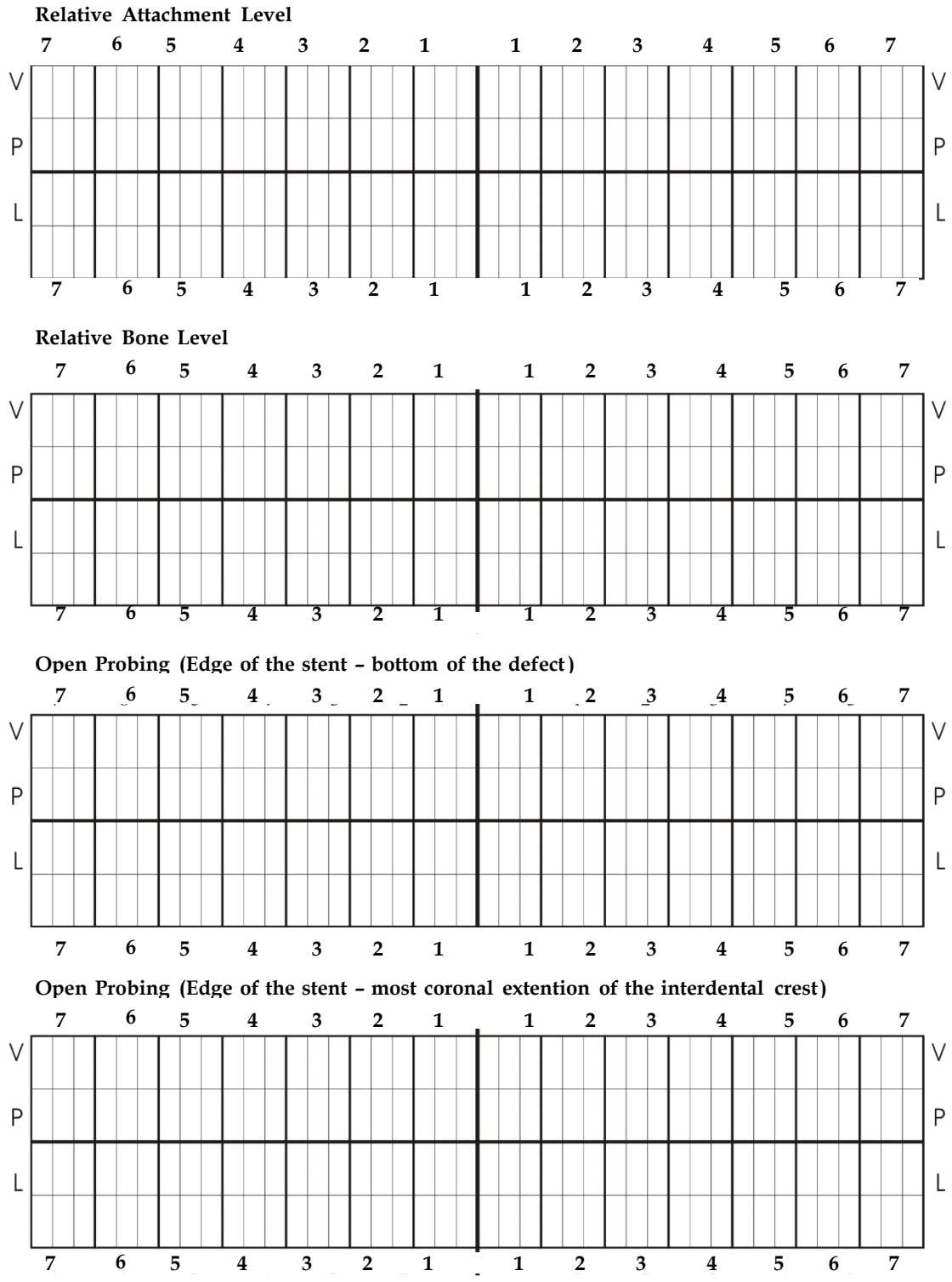
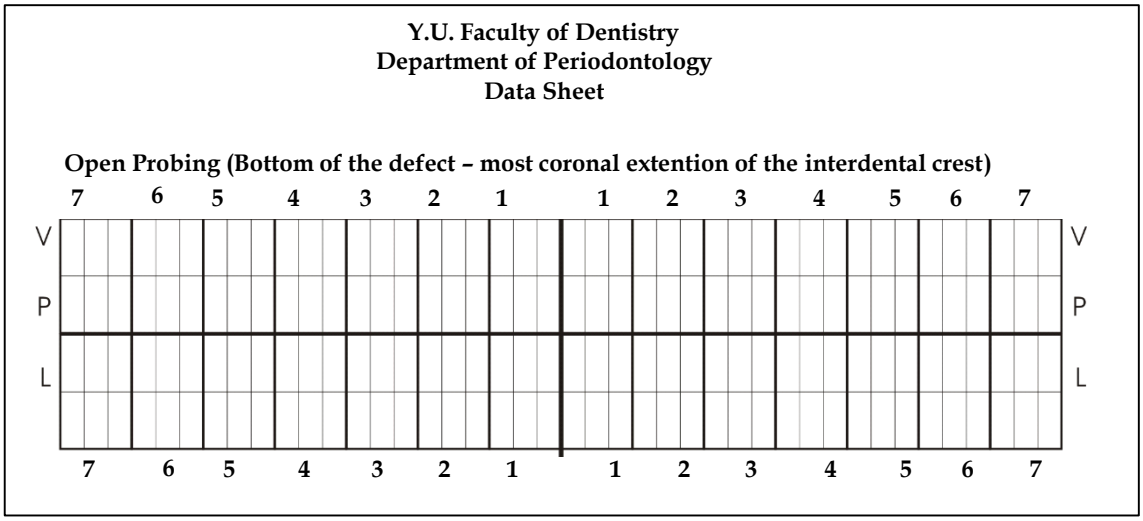


Figure 3. Clinical indices and measurements.



3+2+1-Wall	3+2-Wall	3+1-Wall	3-Wall	2+1-Wall	2-Wall

Figure 4. Clinical indices and measurements.

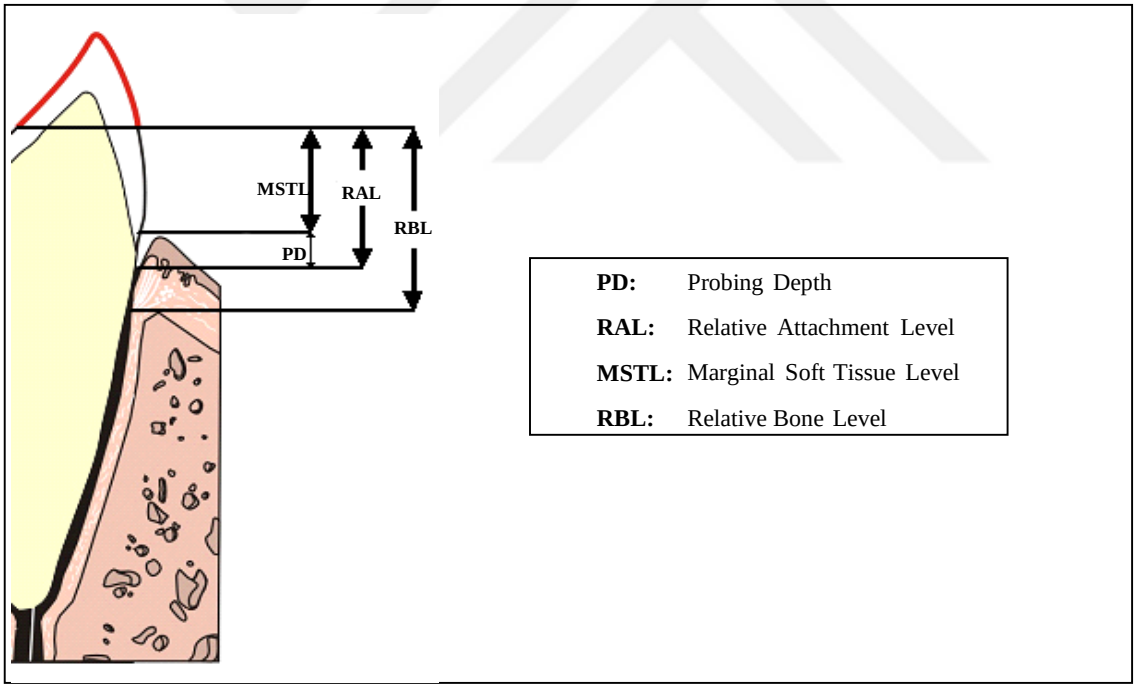


Figure 5. Clinical measurements.

3.5. Intraoperative Measurements

During the surgery, after elevation of the mucoperiosteal flaps and removal of the granulation tissues, intrabony defect depths (IBDD) were calculated with the following formula (109);

1. Distance from the apical edge of the stent to the bottom of the intrabony defect (A);
2. Distance from the apical edge of the stent to the top of the intrabony defect (B);
3. A – B = C (IBDD) (Figure 6).

Following the calculation of IBDD, defects were classified by the number of osseous walls, and recorded along with the other surgical notes (Figures 3, 4).

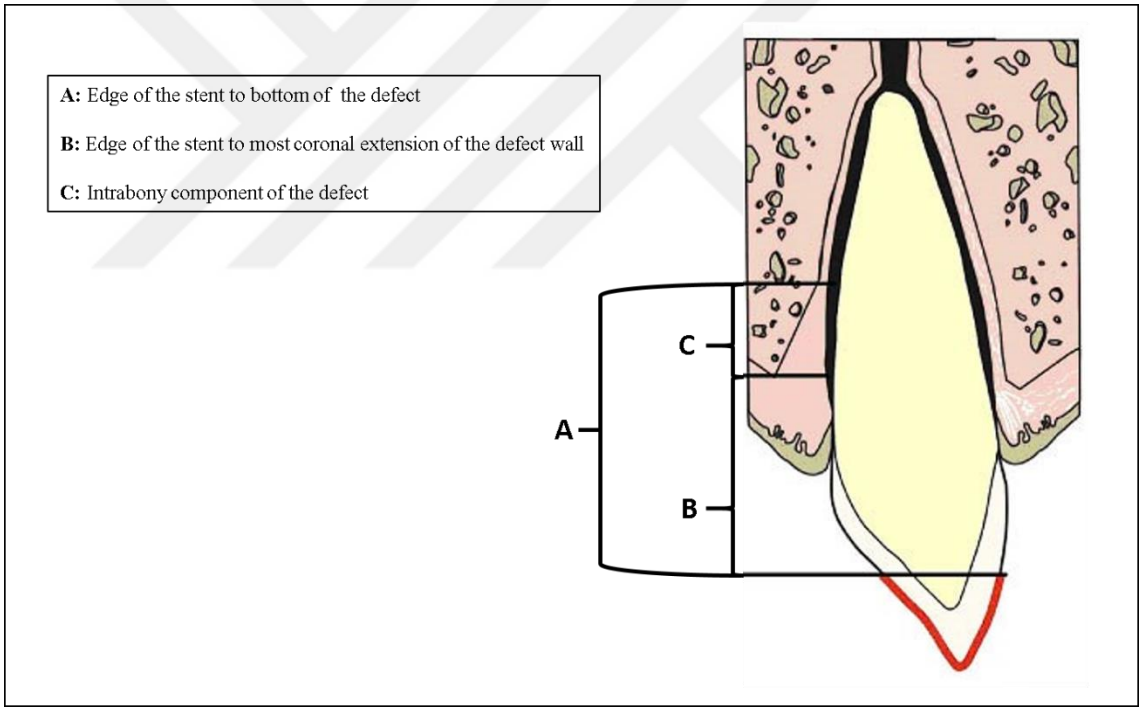


Figure 6. Intrabony defect measurements.

3.6. Radiographic Method and Radiographic Bone Level Measurement

For each patient, standard periapical radiographies were taken before and 12 months after the surgeries. Each periapical radiography was taken using the same type of periapical film[4] and same exposure time. Measurements were done on the radiographies using X-ray grids[5]. Radiographic bone level (Rad BL) was measured as the distance between the deepest site of the defect (DSD) and apex of the related tooth (110).

3.7. Test Material

The bone graft material used in the study was BDX[6] (Figure 7). The other material, PRF, was prepared from patient's own blood using 10ml blood collection tubes[7], blood collection kit[8], table top centrifuge[9] and PRF Box[10] (Figure 8.a-d).

Figure 7. BDX.

[4] **Kodak,** Ultra Speed, Readymatic, X-Omet, France.
[5] **X-ray Grid**, Meyer Haake, GmbH, Oberursel, Germany.
[6] **Bio-Oss® Geistlich Pharma AG**, Wolhusen, Switzerland.
[7] **VACUETTE 10 ml blood collection**, Greiner bio-one, North Carolina, USA.
[8] **BD Vacutainer®**, Becton, Dickinson and Company, Plymouth, USA.
[9] **Hettich EBA 20 Centrifuge**, Tutlingen, Germany.
[10] **PRF box**, Istanbul, Turkey.

a

b

c

D

Figure 8. Materials for PRF preparation. **a.** 10ml Blood collection tubes, **b.** Blood collection kit, **c.** Table top centrifuge, **d.** PRF box.

3.7.1. Preparation of Platelet Rich Fibrin

Intravenous blood was collected from the antecubital vein of the patients into 10 ml glass coated plastic tubes without anticoagulant coating and were immediately centrifuged at 3000 rpm for 10 minutes. Processed tubes contained 3 layers from top to bottom; platelet poor plasma, fibrin clot and red corpuscles, respectively. The fibrin clot was removed from the tube and the attached red blood cells were separated using scissors. The clots were placed on a perforated plate in the PRF box and compressed by a cover to create a fibrin membrane (Figure 9.a-d).

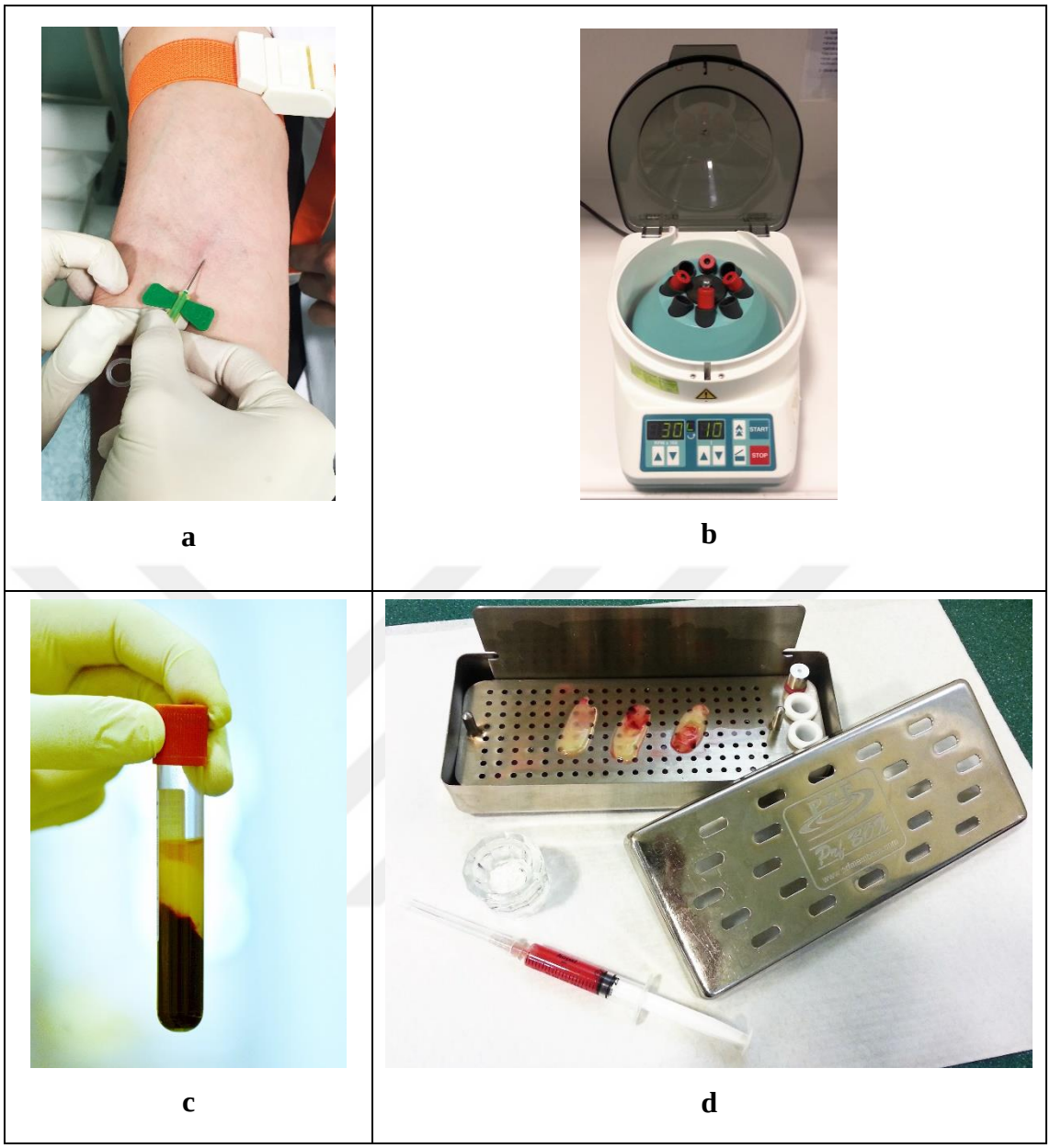


Figure 9. PRF preparation. **a.** Blood collection, **b.** Centrifugation process, **c.** PRF inside the collection tube, **d.** Compression of PRF.

3.8. Surgical Procedure

For each patient, operative procedure either in maxilla or mandible was performed in one session. If necessary, the surgery of the remaining jaw was performed 1 month after the first one. Following local anesthesia[11], a modified intrasulcular incision was performed. It was placed approximately 0.5 mm from the gingival margin aiming to remove the pocket epithelium. Mucoperiosteal flaps were then reflected. Granulation tissues adherent to the alveolar bone were removed to provide full access and visibility to

[11] **Ultracain DS Fort 2 ml**, Aventis Pharma, Istanbul, Turkey.

the root surfaces. Any subgingival calculus was removed and the root surfaces were gently scaled and planed with hand and ultrasonic instruments. No osseous recontouring was performed. Surgical sites were rinsed with sterile saline and bleeding was controlled. Surgical site was than isolated and contamination with blood and/or saliva was prevented. Defect sites were filled with BDX mixed with saline solution (Figure 10) or with BDX mixed with PRF exudate which then was covered with PRF membrane (Figure 11.a-b). Care was taken not to overfill the defects. Flaps were primarily sutured using 5-0 propylene sutures[12]

Figure 10. Materials for BDX group. BDX mixed with saline.

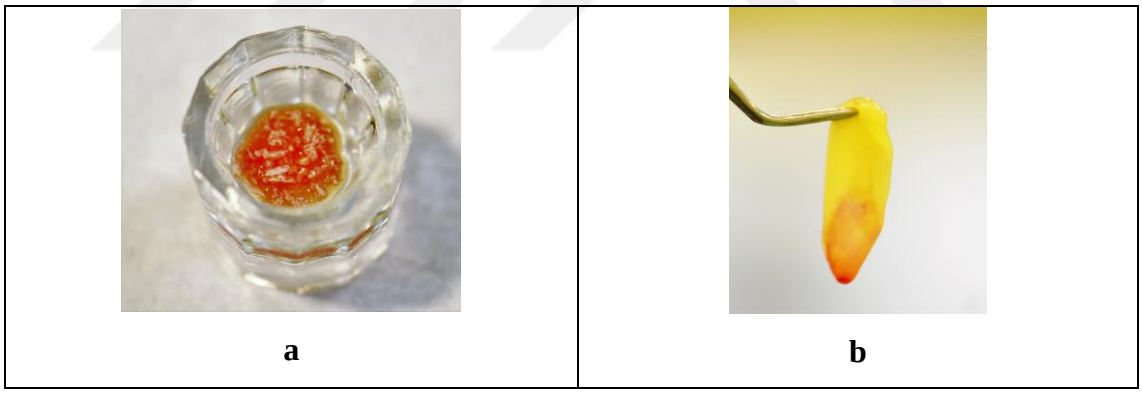


Figure 11. Materials for PRF + BDX group. **a.** BDX mixed with PRF exudate, **b.** PRF membrane.

3.9. Post-Operative Infection Control

Following surgery, patients were prescribed systemic amoxicillin + clavulonic acid[13] (1000 mg, 2X1) for 1 week and chlorhexidine gluconate containing oral rinse[14] (0.2%,

[12] **Prolene 5-0 sutures, Ethicon,** Johnson & Johnson, New Jersey, USA.
[13] **Augmentin BID 1000 mg**, GlaxoSmithKline Pharmaceuticals Istanbul, Turkey.
[14] **Klorhex Oral Rinse % 0.2,** Drogsan Pharmaceuticals, Istanbul, Turkey.

2X1) for 4 weeks. If necessary, analgesics[15] (Naproxen sodium, 550 mg, 2X1) were prescribed.

3.10. Post-Operative Care

Patients were asked not to use any interdental cleaning devices on the surgical sites until the sutures were removed. At 1 week, professional tooth cleaning and polishing was performed on the surgical site. Care was taken to stay away from the free gingival margin. For the first 2 weeks, patients were asked to gently brush the teeth surfaces and wipe the gingiva with a sterile gauze damped with saline solution. Sutures were removed at 2 weeks. After suture removal, patients were instructed to use interdental devices on the surgical sites. Professional tooth cleaning and polishing was performed every week from post-operative 2 weeks to 2 months, every 2 weeks from post-operative 2 months to 3 months, and every 4 weeks from post-operative 3 months to 12 months. Oral hygiene instructions were reinforced when necessary (111). During the 12-month follow-up period, no probing and/or subgingival scaling was performed.

3.11. Data Evaluation

Data obtained before and 12 months after the surgery was evaluated in regards to plaque accumulation, gingival bleeding, PD reduction, attachment gain, gingival recession and clinical and radiographic bone gains. Mean values, standard deviations (SD) and median values were calculated for all parameters to be used to evaluate the results of the study. If a patient had more than one defect, the values of the defect sites were taken as a mean and all data were recorded at patient level.

Plaque index values were evaluated as full mouth (4 points) and interproximal measurements (2 points). Full mouth measurements were calculated as the mean value of mesial, mid-coronal and distal points for buccal side together with mid-coronal point for the lingual side. Interproximal measurements were calculated as the mean value of mesial and distal points of the buccal aspect (Figure 2).

Sulcus bleeding index values were evaluated as full mouth (6 points) and interproximal measurements (4 points). Full mouth measurements were calculated as the mean value of mesial, mid-coronal and distal points for both the buccal and lingual sides. Interproximal measurements were calculated as the mean value of mesial and distal points for both the buccal and lingual sides (Figure 2).

[15] **Apranax Forte Tablets 550 mg**, Abdi Ibrahim, Istanbul, Turkey.

Probing depth, MSTL, RAL, RBL and Rad BL were evaluated with regards to DSD measurements. Deepest site of the defect measurements (1 point) were taken as the deepest value from either buccal or lingual interproximal area neighboring the defect site (Figures 2, 3).

3.12. Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics 22.0[16] program. Intra-examiner calibration was performed using intraclass correlation coefficient. Evaluation of age and gender with respect to the groups were performed using Student's t-test and Continuity (Yates) correction, respectively. For the clinical parameters, mean values, standard deviations and median values were calculated. Parameters which did not present a normal distribution were evaluated via the Mann Whitney U-test for the intergroup differences and via the Wilcoxon Sign test for the intragroup differences. Primary outcome variable was CAL whereas remaining parameters were considered as secondary outcome variables. The power analysis results revealed that, minimum 7 subjects per group were necessary to obtain 80 % statistical power and $\alpha=0.05$ with $\Delta=1.58$, standard deviation (SD): 1 (23). Significance was set at $p < 0.05$.

[16] **IBM SPSS Statistics 22**, New York, USA.

4. RESULTS

4.1. Demographic Results / Defect Types and Distribution

The study was conducted on 20 patients, 11 female and 9 male, diagnosed as advanced chronic periodontitis. First group of patients treated using BDX presented with 49 intrabony defects with mean IBDD of 4 ± 1.17 (4) mm, whereas second group of patients treated using PRF + BDX presented with 43 intrabony defects with mean IBDD of 4.05 ± 0.79 (4) mm. Defects which did not meet the inclusion criteria according to the clinical measurements following initial periodontal therapy or during surgery were excluded. A total of 92 defects, consisting of 4 3+2+1-wall, 6 3+2-wall, 6 3+1-wall, 7 3-wall, 27 2+1-wall and 42 2-wall in morphology were evaluated. Defects were distributed as 17 incisor/canine, 25 premolar and 50 molar teeth. The distribution of the defects among patients are presented in Table 1a, whereas the distribution according to morphology and localization are presented in Tables 1b and 1c, respectively.

Table 1a. Defect distribution among patients.

Number of Patient	**1**	**3**	**5**	**6**	**4**	**1**
Number of Defects	**2**	**3**	**4**	**5**	**6**	**7**

Table 1b. Defect distribution according to morphology.

			Number of Defect Walls			
BDX	**3+2+1 Wall**	**3+2 Wall**	**3+1 Wall**	**3 Wall**	**2+1 Wall**	**2 Wall**
Number of Teeth	3	2	4	4	17	19
PRF + BDX	**3+2+1 Wall**	**3+2 Wall**	**3+1 Wall**	**3 Wall**	**2+1 Wall**	**2 Wall**
Number of Teeth	1	4	2	3	10	23

Table 1c. Defect distribution according to localization.

		Defect Localization	
BDX	Incisor / Canine	Premolar	Molar
Number of Teeth	6	16	27
PRF + BDX	Incisor / Canine	Premolar	Molar
Number of Teeth	11	9	23

4.2. Clinical Results

During the post-operative healing period, no signs of infection such as pus or abscess formation were observed. Patients did not develop any adverse reactions to the materials used and no side effects were observed regarding systemic antibiotic use. Discoloration of teeth and tongue was seen due to chlorhexidine containing antibacterial oral rinse. Intraoral images and radiographies of a patient from each treatment group are presented in Figure 12a-i and Figure 13a-h.

Mean values, SD and median values of the clinical indices and measurements regarding the two treatment groups are presented in Tables 2-8.

4.2.1. Plaque Index

Evaluation of full mouth and interproximal PI values revealed a statistically significant decrease from baseline to 12 months for both treatment groups ($p < 0.01$). The comparison of 12-month full mouth and interproximal PI values of two treatment groups were found to be statistically insignificant ($p > 0.05$). Mean values, SD and median values including intra/inter group comparisons regarding PI at baseline and 12 months for both treatment groups are presented in Table 2.

Table 2. Baseline and 12-month PI values.

		BDX Mean ± SD (median)	PRF + BDX Mean ± SD (median)	[1]p
PI Full Mouth	**Baseline**	0.33 ± 0.06 (0.33)	0.35 ± 0.08 (0.34)	**0.649**
	12 Months	0.19 ± 0.07 (0.19)	0.20 ± 0.06 (0.19)	**0.970**
	Difference	0.13 ± 0.09 (0.17)	0.15 ± 0.05 (0.15)	**0.909**
	P	**0.007****	**0.005****	
PI Interproximal	**Baseline**	0.45 ± 0.05 (0.44)	0.46 ± 0.08 (0.45)	**0.820**
	12 Months	0.24 ± 0.05 (0.23)	0.27 ± 0.04 (0.28)	**0.161**
	Difference	0.21 ± 0.08 (0.2)	0.19 ± 0.08 (0.19)	**0.520**
	[2]p	**0.005****	**0.005****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

4.2.2. Sulcus Bleeding Index

Evaluation of full mouth and interproximal SBI values revealed a statistically significant decrease from baseline to 12 months for both treatment groups ($p < 0.01$). The comparison of 12-month full mouth and interproximal SBI values of two treatment groups were found to be statistically insignificant ($p > 0.05$). Mean values, SD and median values including intra/inter group comparisons regarding SBI at baseline and 12 months for both treatment groups are presented in Table 3.

Table 3. Baseline and 12-month SBI values.

		BDX Mean ± SD (median)	**PRF + BDX Mean ± SD (median)**	**[1]p**
SBI Full Mouth	**Baseline**	0.4 ± 0.08 (0.42)	0.42 ± 0.09 (0.41)	**0.849**
	12 Months	0.22 ± 0.05 (0.24)	0.21 ± 0.06 (0.21)	**0.344**
	Difference	0.18 ± 0.07 (0.17)	0.21 ± 0.09 (0.21)	**0.595**
	[2]p	**0.005****	**0.005****	
SBI Interproximal	**Baseline**	0.52 ± 0.08 (0.5)	0,51 ± 0,08 (0.5)	**0,733**
	12 Months	0.31 ± 0.06 (0.31)	0,27 ± 0,05 (0.27)	**0,172**
	Difference	0.21 ± 0.09 (0.21)	0,24 ± 0,07 (0.24)	**0,595**
	[2]p	**0.005****	**0.005****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

4.2.3. Marginal Soft Tissue Level

Evaluation of MSTL values revealed a statistically significant apical migration of free gingiva from baseline to 12 months resulting in gingival recession for both treatment groups ($p < 0.01$). Differences between two treatment groups for gingival recession was found to be statistically significant in favor of the PRF + BDX group ($p < 0.01$). Mean values, SD and median values including intra/inter group comparisons regarding MSTL at baseline and 12 months for both treatment groups are presented in Table 4.

Table 4. Baseline and 12-month MSTL values.

		BDX Mean ± SD (median)	PRF +BDX Mean ± SD (median)	[1]p
MSTL (DSD)	Baseline	5.23 ± 1.75 (5)	4.98 ± 1.35 (5)	
	12 Months	6.02 ± 1.68 (6)	5.3±1.3 (5)	
	Difference	0.79 ± 0.56 (1)	0.33±0.52 (0)	0.001**
	[2]p	0.001**	0.001**	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

4.2.4. Probing Depth

Evaluation of PD values revealed a statistically significant PD reduction from baseline to 12 months for both treatment groups ($p < 0.01$). Difference between two treatment groups for PD reduction at 12 months was found to be statistically insignificant ($p > 0.05$). Mean values, SD and median values including intra/inter group comparisons regarding PD at baseline and 12 months for both treatment groups are presented in Table 5.

Table 5. Baseline and 12-month PD values.

		BDX Mean ± SD (median)	PRF + BDX Mean ± SD (median)	[1]p
PD (DSD)	Baseline	5.98 ± 1.20 (6)	6.28 ± 1.03 (6)	**0.088**
	12 Months	2.98 ± 1.18 (3)	2.93 ± 1.33 (3)	**0.611**
	Difference	3.0 ± 1.41 (3)	3.35 ± 1.25 (3)	**0.186**
	[2]p	**0.001****	**0.001****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

4.2.5. Relative Attachment Level

Evaluation of RAL values revealed a statistically significant attachment gain from baseline to 12 months for both treatment groups ($p < 0.01$). Difference between two treatment groups for attachment gain at 12 months was found to be statistically significant in favor of the PRF + BDX group ($p < 0.01$). Mean values, SD and median values including intra/inter group comparisons regarding RAL at baseline and 12 months for both treatment groups are presented in Table 6.

Table 6. Baseline and 12-month RAL values.

		BDX Mean ± SD (median)	**PRF + BDX Mean ± SD (median)**	[1]**p**
RAL (DSD)	**Baseline**	11.09 ± 1.99 (10.5)	11.26 ± 1.59 (11)	
	12 Months	8.94 ± 1.57 (9)	8.23 ± 1.99 (8)	
	Difference	2.15 ± 1.35 (2)	3.02 ± 1.26 (3)	**0.002****
	[2]**p**	**0.001****	**0.001****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

4.2.6. Relative Bone Level

Evaluation of RBL values revealed a statistically significant clinical bone gain from baseline to 12 months for both treatment groups ($p < 0.01$). Difference between two treatment groups for clinical bone gain values were found to be statistically significant in favor of the PRF + BDX group ($p < 0.01$). Mean values, SD and median values including intra/inter group comparisons regarding RBL at baseline and 12 months for both treatment groups are presented in Table 7.

Table 7. Baseline and 12-month RBL values.

		BDX Mean ± SD (median)	**PRF + BDX Mean ± SD (median)**	**[1]p**
RBL (DSD)	**Baseline**	12.41 ± 1.96 (12)	12.65 ± 1.63 (13)	
	12 Months	10.24 ± 1.79 (10)	9.91 ± 2.01 (10)	
	Difference	2.10 ± 1.2 (2)	2.74 ± 1 (3)	**0.003****
	[2]p	**0.001****	**0.001****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01

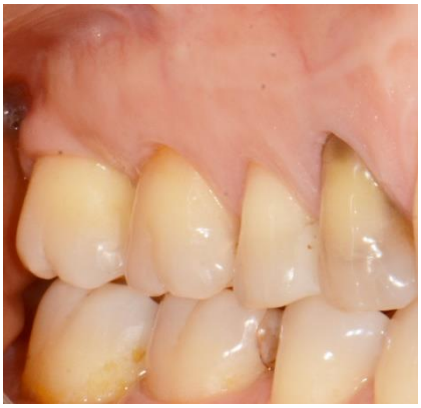
4.2.7. Radiographic Bone Level

Evaluation of Rad BL values revealed a statistically significant radiographic bone gain (Rad BG) from baseline to 12 months for both treatment groups ($p < 0.01$). Difference between two treatment groups for Rad BG were found to be statistically significant in favor of PRF + BDX group ($p < 0.01$). Mean values, SD and median values including intra/inter group comparisons regarding Rad BL at baseline and 12 months for both treatment groups are presented in Table 8.

Table 8. Baseline and 12-month Rad BL values.

		BDX Mean ± SD (median)	**PRF + BDX Mean ± SD (median)**	**[1]p**
Rad BL (DSD)	**Baseline**	6.06 ± 0.77 (6)	6.23 ± 0.75 (6)	**0.285**
	12 Months	8.31 ± 1.06 (8)	8.86 ± 1.13 (9)	**0.009**
	Difference	2.24 ± 1.03 (2)	2.63 ± 0.98 (3)	**0.031***
	[2]p	**0.001****	**0.001****	

[1] Mann Whitney U test [2] Wilcoxon sign test *p<0.05 ** p<0.01



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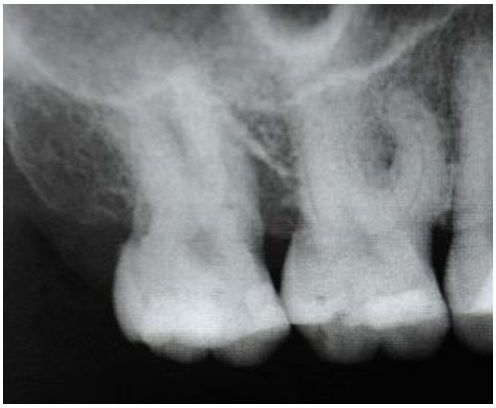

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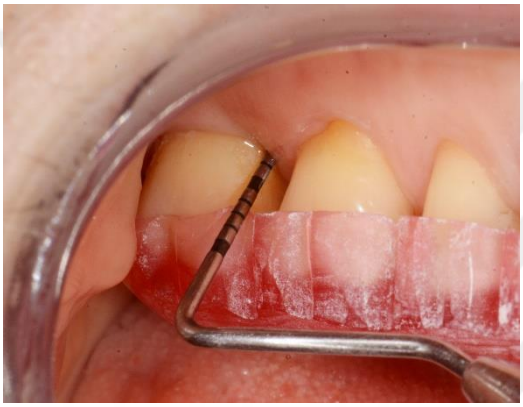

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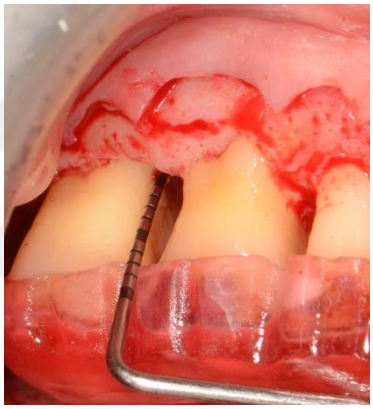

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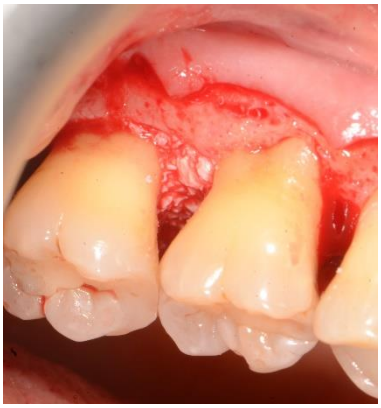

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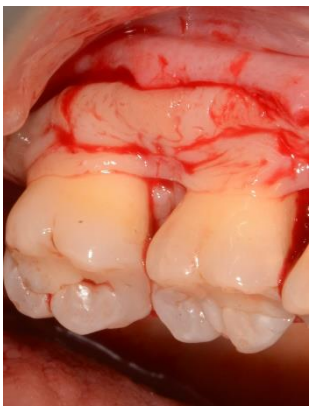

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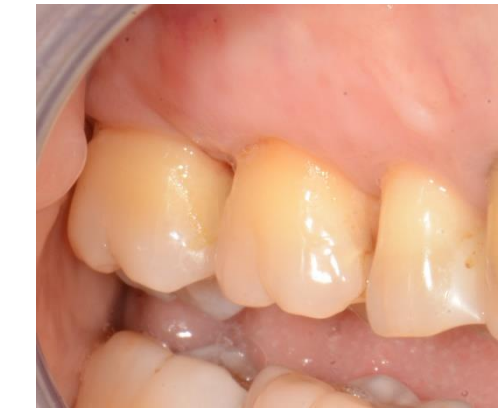

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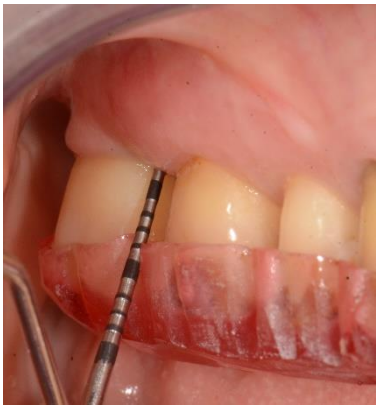

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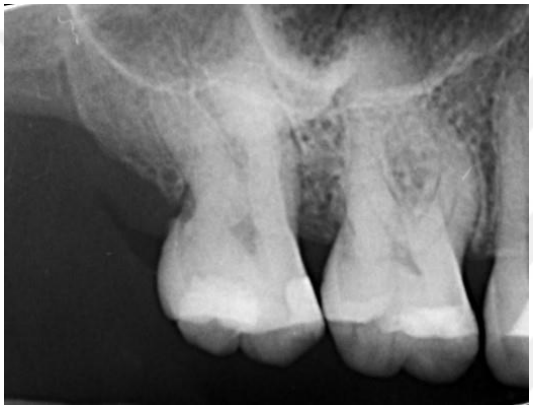

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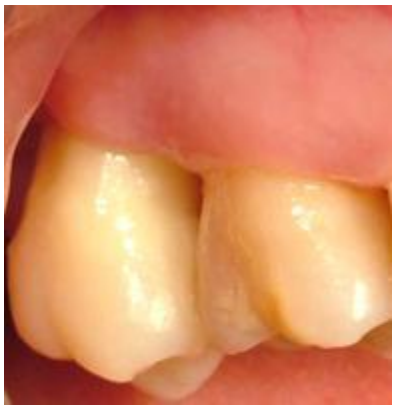
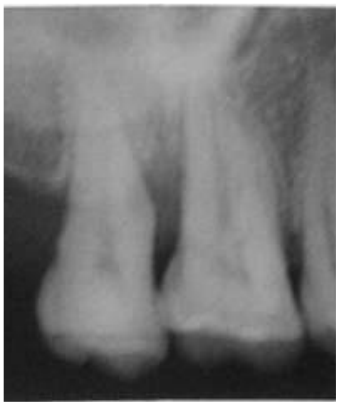
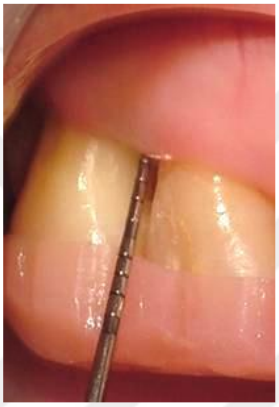
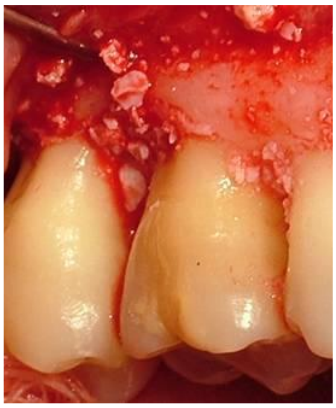
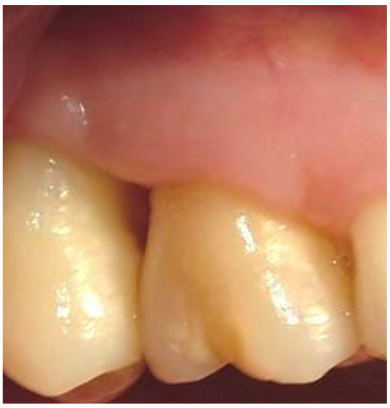
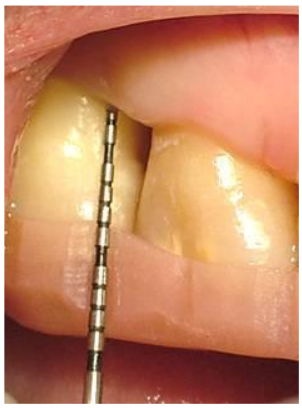
Figure 12. A patient from PRF + BDX group. **a.** Pre-operative clinical view, **b.** Pre-operative radiographic view, **c.** Pre-operative clinical measurements, **d.** Intraoperative measurements, **e.** Application of BDX into the defect, **f.** Application of PRF membrane over the defect, **g.** 12-month clinical view, **h.** 12-month clinical measurements, **i.** 12-month radiographic view.

 **a**	 **b**
 **c**	 **d**
 **e**	 **f**



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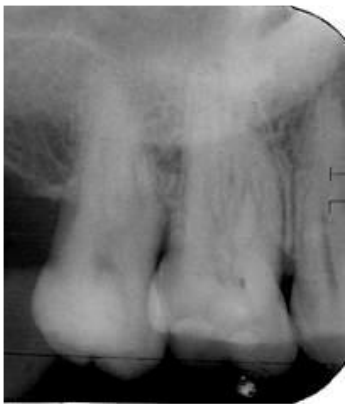

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Figure 13. A patient from BDX group. **a.** Pre-operative clinical view, **b.** Pre-operative radiographic view, **c.** Pre-operative clinical measurements, **d.** Intraoperative measurements, **e.** Application of BDX into the defect, **f.** 12-month clinical view, **g.** 12-month clinical measurements, **h.** 12-month radiographic view.

5. DISCUSSION and CONCLUSION

The aim of this randomized controlled clinical study was to evaluate clinical and radiographic effects of PRF combined with BDX to the use of BDX alone in the treatment of periodontal intrabony defects.

At 12 months, both treatment groups revealed significant clinical and radiographic improvements when compared to baseline ($p < 0.01$). Regarding the DSD, following changes in clinical and radiographic parameters were observed at 12 months for PRF + BDX and BDX groups respectively; a mean PD reduction of 3.35 ± 1.25 (3) mm and 3.0 ± 1.41 (3) mm, attachment gain of 3.02 ± 1.26 (3) mm and 2.15 ± 1.35 (2) mm, gingival recession of 0.33 ± 0.52 (0) mm and 0.79 ± 0.56 (1) mm, clinical bone gain of 2.74 ± 1 (3) mm and 2.10 ± 1.2 (2) mm and radiographic bone gain of 2.63 ± 0.98 (3) mm and 2.24 ± 1.03 (2) mm. Intergroup analysis demonstrated significant attachment gain, gingival recession, clinical bone gain ($p < 0.01$) and radiographic bone gain ($p < 0.05$) in favor of the PRF + BDX group.

The regenerative periodontal treatment outcomes depend on the patient related factors as well as the used biomaterials and surgical techniques. Studies on the effects of periodontal treatment modalities suggest that systemic problems such as uncontrolled diabetes, immune deficiencies or autoimmune diseases may affect the treatment outcomes (112). Medications such as corticosteroids and immune modulators which alter the tissue response or increase the risk of infection may affect the results of regenerative periodontal treatment (113). In order to eliminate the possible effects of these diseases and medications, and to obtain a homogenous patient population, systemically healthy individuals diagnosed as advanced chronic periodontitis were enrolled in the present study.

Defect characteristics such as, defect angle, depth, number of walls and the amount of healthy periodontal tissues play a crucial role on the outcome of regenerative periodontal treatment (44, 113). Deep and narrow defects exhibit a higher amount of attachment and bone gain compared to narrow and shallow defects (60, 114, 115). Source of progenitor cells occupying the defect site decreases with non-contained bony walls, and it was stated that cells from periodontal ligament were not able to migrate into the defect site (116). Three walled defects were shown to have greatest potential for regeneration and in contrast, 1 or 2 wall intrabony defects were found to have a lower potential (117). On the other hand, there are some studies indicating that there is no connection between attachment gain and defect morphology (118). Based on this

information, defects with 3+2+1, 3+2, 3+1, 3, 2+1 and 2 wall morphology presenting intrabony component ≥ 3 mm were included in the present study.

One principle of regenerative periodontal treatment is to prevent any gingival recessions by preserving the soft tissues while reducing the pocket depths (70, 112). The amount of keratinized tissue following grafting of the defect is critical for primary closure of the surgical site. Primary closure creates the basis for preventing post-operative infection and yields greater healing outcomes thus effects the success of regeneration. Most frequently used incision for application of PRF is the intrasulcular incision (23-31). In this study, intrasulcular incisions were used to eliminate any differences which may occur due to incision technique.

Following periodontal treatment, it takes time for the hard and soft tissue parameters to re-establish (42, 119). Therefore, time period for evaluating the treatment outcomes is critical. Time needed for new attachment and bone formation was depicted to be between 6 and 12 months (42, 119). Regarding the literature on the use of BDX in the treatment of intrabony periodontal defects, it was stated that post-operative radiographic and clinical evaluation periods were either 6 or 12 months (48, 58). When literature on PRF application is evaluated, post-operative evaluation periods of 6, 9 and 12 months can be observed (20-31). Based on these studies and also considering the low resorption rate of BDX (84), post-operative evaluation of this study was done at 12 months to be able to evaluate the long term effects of PRF + BDX combination.

Smoking is a major risk factor which impairs the tissue response to periodontal treatment by delaying wound healing and increasing attachment and bone loss (64). It was shown that, nicotine in the peripheral blood impairs neutrophil function, decreases antibody production and, by binding to fibroblasts, impairs the cell functions such as collagen synthesis (120). Local effects are vasoconstriction, decrease in gingival blood flow and decrease in gingival tissue oxygen levels leading to accumulation of metabolic waste products (120). Clinical, epidemiologic and in vitro studies showed that smoking alters the host response and negatively effects periodontal health (121). It was also reported that smoking masks the periodontal infection by reducing gingival bleeding (122). This reduction in bleeding is correlated with increased tissue keratinization and decreased tissue perfusion due to vasoconstriction (123). Factors causing alveolar bone loss was investigated in a study with a follow-up period of 10 years, and a positive correlation between smoking and alveolar bone loss was found (124). Tonetti et al. (64) conducted a study investigating the effects of smoking on GTR in periodontal intrabony

defects. At the end of 12 months, it was shown that non-smokers gained 3.1 mm more attachment compared to smokers. This difference was found to be statistically significant. In another study, Yılmaz et al. (125) investigated the effect of smoking on treatment outcomes of PRP + BDX application in intrabony defects. After 12 months, all clinical parameters for non-smokers were found to be better than smokers in terms of PD reduction (4.63 mm vs 3.97 mm), attachment gain (4.06 mm vs 3.26 mm) and bone gain (3.63 mm vs 3.26 mm). In this study, based on well documented negative effects of smoking on regenerative treatment outcomes, only non-smoker patients were included.

Preparation of the PRF was done according to Choukroun et al. (91). Blood samples were collected from antecubital veins of the patients in 10ml glass coated plastic tubes without anticoagulants. Samples were immediately centrifuged at 3000 rpm for 10 minutes. Most of the studies on the use of PRF in the treatment of intrabony defects were conducted using the protocol of Choukroun et al. (21, 22, 24-26, 28-30). However there are some studies conducted with altered time, force or rpm for the preparation (20, 23, 27, 31).

Another factor effecting the periodontal treatment outcome is wound stabilization and infection control. The studies evaluating the effects of regenerative periodontal treatments suggested regular recall sessions to prevent microbial dental biofilm influencing the treatment outcomes (126). In our study, patients were recalled for control and professional tooth cleaning/polishing. Sutures were removed at 2 weeks. Patients were instructed not to use interproximal cleaning devices to prevent soft tissue trauma and destabilization of the wound until the sutures were removed. At this time period, professional tooth cleaning was performed with one week intervals and patients were instructed to use 0.2 % chlorhexidine containing oral rinse twice a day for 4 weeks to prevent microbial dental biofilm to negatively affect the tissue healing. During the 12-month follow-up period, patients were recalled for once a week for the first 2 months, once every 2 weeks up to 3 months and once a month until 12-month period was completed.

Primary etiologic factor for chronic periodontitis is microbial dental biofilm (127). It is a well-known fact that microbial dental biofilm negatively influences the periodontal treatment outcomes (113, 115). It was shown that clinical outcomes of regenerative treatments are better in patients with low PI (128). Decreasing the microbial load and managing the periodontal pathogens at pre-operative phase by excellent plaque control eliminates the possible negative effects on healing following periodontal surgery (129).

For this reason, all patients included in the study received initial periodontal therapy, oral hygiene levels were evaluated and instructions were reinforced at every recall session. A 3-month time period was set for evaluation of initial periodontal therapy. In this time interval, patients who could not maintain excellent oral hygiene status were excluded and were not proceeded to surgical phase. By doing so, any possible differences on clinical outcomes which may arise from insufficient oral hygiene levels were prevented.

Microbial dental biofilm quantity and oral hygiene levels were assessed using Silness and Löe (107) PI in all treated patients. This index measures the amount of plaque that is in contact with marginal gingival tissues. In both treatment groups, evaluation of full mouth and interproximal PI values revealed a statistically significant decrease from baseline to 12 months ($p < 0.01$) (Table 2). PI differences between the two treatment groups at baseline and 12 months were found to be statistically insignificant ($p > 0.05$) (Table 2). These findings indicate that all patients in both treatment groups were able to maintain excellent oral hygiene status throughout the study period, thus eliminating the possibility of any negative effects on treatment outcomes due to less than optimal plaque control levels (130).

As part of the initial periodontal therapy, SRP was performed to all patients. Debridement of the teeth and root surfaces resulted in reduction of periodontal infection, therefore, minimized the clinical signs of periodontal tissue inflammation. Bleeding is a cardinal symptom of periodontal infection and can be used to evaluate the level of tissue inflammation. In our study, SBI was used to assess the periodontal status (108). With this index, gingival bleeding, tissue edema, changes in color were evaluated. Analysis of full mouth and interproximal SBI values revealed a statistically significant decrease from baseline to 12 months for both of the treatment groups ($p < 0.01$) (Table 3). Difference between the two treatment groups for full mouth and interproximal SBI values at baseline and 12 months were found to be statistically insignificant ($p > 0.05$) (Table 3). These results ensure that the treatment outcomes were not negatively influenced by clinical signs of infection. On the other hand, these results are correlated with the decrease of PI values, indicating elimination of microbial dental biofilm to be critical for periodontal infection control.

Methods for evaluating the effects of regenerative periodontal treatments are periodontal probing, radiographic data, re-entry and histologic examinations (131). True outcome of regenerative periodontal treatment may only be evaluated with histologic examinations. However, due to scientific and ethical concerns, it is not possible to

perform on all patients. Therefore, treatment outcomes should be evaluated using soft and hard tissue measurements. Soft tissue parameters evaluate the changes in pocket depths, attachment levels and gingival recessions. Hard tissue parameters consist of bone level measurements. The regenerative outcome of the treatment can be assessed by the above-mentioned measurements confirming; elimination of periodontal infection together with PD reduction as well as attachment and bone gains (132).

In this study, all soft and hard tissue measurements were done using a periodontal probe together with occlusal acrylic stents. The stents were individually fabricated for each patient with grooves on lingual and buccal surfaces for probe placement. This way, reproducible and reliable probe position and angulation can be performed at baseline and 12 months. The apical edge of the stent was used as a relative reference point for MSTL, RAL and RBL measurements. In various studies, the use of occlusal stents were shown to be a dependable method for obtaining reliable and standardized measurements (133, 134). However, errors may occur due to alterations in probing force and the presence of gingival inflammation. Presence of inflammation may allow the probe tip to penetrate the connective tissue attachment, thus overestimate the attachment loss. On the other hand, elimination of inflammation following the treatment could be misinterpreted as regeneration (135). To eliminate any errors which may occur due to different examiners, all the measurements were performed by a single calibrated clinician.

MSTL measurements performed at baseline and after periodontal treatment reveal the amount of gingival recession. The apical migration of the soft tissues may occur due to resolution of inflammation and/or resective periodontal incisions aimed at reducing probing pocket depths. On one hand, gingival recession leads to PD reduction which is a favorable treatment outcome, on the other hand, negatively influences attachment gain thus impedes periodontal regeneration (112). An unfavorable result of gingival recession is the exposition of root surfaces, creating aesthetic problems and possible hypersensitivity. In this study, sulcular incisions were used to preserve the soft tissues and minimize gingival recessions. At 12 months, PRF + BDX group exhibited 0.33 ± 0.52 (0) mm of gingival recession compared to 0.79 ± 0.56 (1) mm in BDX group ($p < 0.01$) (Table 4). Difference between the two treatment groups was found to be statistically significant in favor of the PRF + BDX group ($p < 0.01$) (Table 4).

Studies conducted on BDX reported gingival recessions ranging from 0 mm to 1.5 mm (58, 59, 61, 63, 68, 90). Gingival recession of 0.79 ± 0.56 (1) mm in BDX group of our study is in accordance with other studies in the literature.

In the literature, there is only one study investigating the effects of PRF + BDX. Lekovic et al. (23) reported the gingival recession to be 0.65 ± 0.59 mm for the buccal and 0.59±0.49 mm for the lingual sites for PRF + BDX group and 1.06 ± 0.42 mm for buccal and 1.12 ± 0.32 mm for the lingual sites for PRF group at 6 months. Although gingival recession was found to be lower for the PRF + BDX group, difference between the two treatment groups was found to be statistically insignificant ($p > 0.05$). In this study, surgical protocol for PRF + BDX group included a mixing minced PRF particles with graft material and covering the defects with PRF membranes which is different than our study where only the PRF membranes were used and graft material was mixed with PRF exudate. The reported result on recession for PRF + BDX group is similar but slightly higher than our finding of 0.33 ± 0.52 (0) mm. This difference may be due to higher resorption rate of PRF particles mixed with the graft material.

On the other hand, there are two other studies conducted with PRF + DFDBA. Agarwal et al. (20) reported 0.47 ± 0.56 mm of gingival recession for PRF + DFDBA and 1.00 ± 0.61 mm for DFDBA group at 12 months. Difference between the two groups was found to be statistically significant ($p < 0.01$). It must be noted that in both treatment groups, PRF membranes were placed to cover the defects and in PRF + DFDBA group, minced PRF pieces were mixed with the graft material in contrast to saline in the DFDBA group.

The other study conducted by Bansal and Bharti (21) reported 0.2 ± 0.422 mm of gingival recession for PRF + DFDBA and 0.4 ± 0.588 mm for DFDBA group at 6 months. Surgical protocol for PRF + DFDBA group consisted of placing a mixture of graft material with minced PRF particles however defects were not covered by PRF membranes. Difference between the two treatment groups was found to be statistically insignificant ($p>0.01$).

The amount of gingival recession observed in PRF + BDX group of our study is in the range of other studies conducted with BDX groups and similar to studies with PRF + DFDBA groups. Differences between the studies can be allocated to defect types, flap thickness and surgical technique as well as the different methods for using the PRF biomaterial at the surgical site (136).

PD is a crucial soft tissue parameter in both the diagnosis of the periodontal diseases and the assessment of the periodontal treatment outcomes. PD is defined as the distance between the free gingival margin and the bottom of the periodontal pocket. An increase or reduction in PD must be evaluated with respect to changes in attachment level

and gingival recession. Following periodontal treatment, PD reduction allows the patient to be able to maintain effective oral hygiene and improves long term stability of treatment outcomes. In this study, PRF + BDX and BDX groups presented with baseline PD of 6.28 ± 1.03 (6) mm and 5.98 ± 1.20 (6) mm, PD reduction of 3.35 ± 1.25 (3) mm and 3.0 ± 1.41 (3) mm, respectively. At the end of 12 months, both treatment groups revealed statistically significant reduction in PD compared to baseline ($p < 0.01$) and no statistically significant difference between the treatment groups were observed ($p > 0.05$) (Table 5). The residual PD for PRF + BDX and BDX groups were found to be 2.93 ± 1.33 (3) mm and 2.98 ± 1.18 (3) mm respectively. This data concludes that, by the end of the study, PD of physiologic levels were obtained thus optimal state for periodontal maintenance and plaque control was created.

Amount of attachment gain involved in PD reduction reflects the regenerative aspect of the treatment outcome. In this regard, attachment level measurements are highly important for the assessment of regenerative treatment procedures (137). Changes in the attachment levels are generally measured as either clinical or relative values. Clinical attachment level is defined as the distance between cemento-enamel junction and bottom of the periodontal pocket (41, 133). However, in some cases, cemento-enamel junction may be located subgingivally and different tooth morphologies/positions may prevent proper probe positioning. These factors decreases the reliability and repeatability of measuring the attachment levels with relation to cemento-enamel junction. Therefore, in our study, apical edges of the acrylic occlusal stents were used as reference points, and the distance between this reference point and the bottom of the periodontal pocket was regarded as RAL. The differences between baseline and 12-month RAL values were evaluated as attachment gain or loss. In this study, evaluation of RAL measurements revealed significant attachment gains for both PRF + BDX and BDX groups compared to baseline ($p < 0.01$) (Table 6). At the end of 12 months, PRF + BDX and BDX groups presented with 3.02 ± 1.26 (3) mm and 2.15 ± 1.35 (2) mm of attachment gain, respectively. The difference between the two treatment groups was found to be statistically significant in favor of PRF + BDX group ($p < 0.01$) (Table 6).

Investigation of the related studies with BDX treatment groups revealed, PD reductions ranging from 2.53 mm to 6.80 mm and attachment gains from 2.31 mm to 7.00 mm (48, 58, 59, 61, 63, 68, 87, 90). In the study of Richardson et al. (48), BDX treated group presented with initial PD of 8.9 mm, PD reduction of 3 mm and attachment gain of 3.6 mm, no data on gingival recession was noted. Despite the baseline PD values being

higher than our study, PD reduction was similar and attachment gain was higher compared to our results. Considering the slow resorption rate of BDX, relatively short 6 month follow-up interval together with inclusion of only 3 and 2 wall intrabony defects may account for the differences observed in the studies. In a case report by Camelo et al. (59), 2 patients were treated as a part of the BDX group. For these two patients, baseline PD values of 9 mm and 10 mm, PD reductions of 4 mm and 6 mm, gingival recessions of 0 mm and 1 mm and attachment gains of 4 mm and 5 mm were reported, respectively. Another study with similar design, including histological examinations, was conducted by Nevins et al. (63). Two patients treated with BDX were reported to have baseline PD of 8 mm and 7 mm, PD reductions of 5 mm and 6 mm and attachment gains of 5 mm and 7 mm. No data on gingival recession were noted. In our study, attachment gains and PD reductions of the BDX treated group are lower than both of these studies. However, difference in number of treated patients prevents a direct comparison. Scabbia and Trombelli (58) evaluated the clinical outcomes of BDX compared to HA. Baseline PD measurement for the BDX group was reported to be 7.5 mm. At 12 months, BDX group exhibited a PD reduction of 4.40 mm and an attachment gain of 4.00 mm. Although this study was conducted with similar follow-up period for evaluation of the outcomes, higher baseline PD values of Scabbia and Trombelli's (58) study may explain the lower PD reduction and attachment gain values observed in our BDX treated group. Hanna et al. (68) evaluated the clinical effects of PRP + BDX compared to BDX. The study included 2- and 3-wall intrabony defects with baseline PD of 7 mm. At 6 months, BDX group showed a PD reduction of 2.53 mm and attachment gain of 2.31 mm. Compared to our results, Hanna et al.'s (68) study presented with higher baseline PD and attachment gain, however lower PD reduction. 6 month follow-up period of this study may account for the observed differences. Ouyang et al (89) conducted a similar study with 12-month follow-up period. Control group presenting with baseline PD of 8.22 mm was treated with BDX. At 12 months, 3.48 mm of PD reduction and 2.85 mm of attachment gain was observed. Döri et al. (90) conducted a study with BDX control group. Baseline measurements revealed an initial PD of 8.5 mm. Clinical measurements were repeated at 12 months after the treatment. Following results were obtained for the BDX group; a PD reduction of 5.3 mm and an attachment gain of 4.7 mm. In case of higher baseline PD measurements, it is well expected to observe higher PD reductions after treatment. When compared to our BDX control group, both of the above-mentioned studies were conducted on patient groups presenting with higher baseline PD measurements. This difference may explain

the lower PD reduction and attachment gain values observed in our study. Varying results obtained from relevant studies may be caused by short follow-up periods, differences in baseline PD measurements, selection of only 2- and 3-wall defects and lower number of treated patients.

The only study with PRF + BDX treatment group was conducted by Lekovic et al. (23). In this study, defects were filled with BDX mixed with minced PRF pieces and were covered with PRF membranes. Baseline PD was reported as 7.94 mm for buccal and 7.88 mm for lingual sites. Measurements were repeated at 6 months and following results were observed; PD reductions of 4.47 mm and 4.29 mm, attachment gains of 3.82 mm and 3.72 mm for buccal and lingual sites, respectively. Results obtained from our study is lower than Lekovic et al.'s (23) results. This difference might be due to higher baseline PD measurements observed in Lekovic et al.'s (23) study. On the other hand, 6 months follow-up period might not be ideal for evaluating the outcomes when a graft material with slow resorption rate such as BDX is used. Also, a direct comparison of the results is not feasible due to different methods used for the application of PRF.

Elgendy et al. (22) used PRF membranes together with nanocrystalline HA grafting material in their test group. At 6 months, following data were obtained; a PD reduction of 3.33 mm and an attachment gain of 3.55 mm. Baseline PD was reported as 6.75 mm, however no data on gingival recession was noted. These results are comparable to our findings with regards to PD reduction, however, attachment gain and baseline PD is higher than our PRF + BDX group. In case of higher baseline PD, greater attachment gain may be expected and short follow-up period used to evaluate regenerative outcomes may account for the differences. On the other hand, use of a different grafting material stands in the way of direct comparison of the results. In another study, Bansal and Bharti (21) applied minced PRF pieces mixed with DFDBA into intrabony defects of their test group and results were evaluated at 6 months. PD reduction of 4.0 mm and attachment gain of 3.4 mm was observed, however, baseline PD was not reported. These results are higher than the results obtained from our PRF + BDX group. In a study conducted by Agarwal et al. (20), patients were either treated with PRF + DFDBA mixture (test) or DFDBA (control). In both groups, defects were covered with a PRF membrane. Baseline PD measurements were reported as 7.13 mm for the test group and 7.12 mm for the control group. At 12 months, test and control groups resulted in PD reductions of 4.15 mm and 3.60 mm, attachment gains of 3.73 mm and 2.61 mm respectively. Control group in this study is similar to our PRF + BDX group in terms of PRF application protocol

where PD reduction was reported to be higher and attachment gain was lower when compared to our results. These differences in the results might be explained by different graft material used and different baseline PD measurements.

Hard tissue parameters such as bone fill and bone gain are used to evaluate the result of the regeneration process taking place inside the intrabony defect, thus the amount of newly formed bone. The most effective method for evaluating bone fill is re-entry surgery. In doing so, regenerated defect site is surgically exposed, visually examined and bone level measurements are taken. Re-entry procedures are very reliable in regards to proving new bone formation however, a second surgery at the treated site creates ethical concerns. An alternative method that yields similar and reliable results is sounding where periodontal probe is advanced inside the periodontal pocket until direct contact with bone is obtained and measurements are taken (138). Thus, in our study, evaluation of new bone formation was done using the sounding method and the obtained measurements were reinforced with radiographic assessments. Sounding data were obtained with the aid of acrylic stents as a guide and relative reference point. Baseline and 12-month data were regarded as RBL values and difference between these values were regarded as bone gain or bone loss. Evaluation of RBL values revealed a statistically significant clinical bone gain from baseline to 12 months for both PRF + BDX (2.74 ± 1 (3) mm) and the BDX (2.10 ± 1.2 (2) mm) groups ($p < 0.01$) (Table 7). Difference between the two treatment groups for clinical bone gain values were found to be statistically significant in favor of the PRF + BDX group ($p < 0.01$) (Table 7).

In the study with BDX treatment group, Richardson et al. (48) evaluated bone fill using the re-entry method. Treated defects were either 2- or 3-wall in morphology. Authors performed the re-entry surgeries 6 months after the treatment and reported 3 mm of bone fill. A direct comparison with the results of our BDX group is not possible due to different evaluation methods. A similar study was conducted by Ouyang et al (89), in which, defects with 2- or 3-walls and intrabony component ≥ 4 mm were treated. At 12 months, BDX group resulted in 2.88 mm of bone gain. Results observed in our BDX group are lower than both of these studies. This differences might be due to lower baseline PD measurements and IBDD of our BDX group as well as the inclusion of 3+2+1, 3+2, 3+1, 3, 2+1 and 2-wall defects in contrast to just 2- or 3-wall defects.

Investigation of the literature on the use of PRF + BDX combination reveals only one study where Levkovic et al. (23) used re-entry method for evaluating defect fill. Re-entry surgeries were performed 6 months after the treatment and authors reported 4.06

mm and 3.94 mm of defect fill for the buccal and lingual sites, respectively. Similar to our study, the treated defects were mainly in 2 wall configuration. Evaluation method prevents a direct comparison of the results however, short follow-up period considering the slow resorption rate of BDX, higher baseline PD values and different application protocol of PRF (minced PRF pieces mixed with BDX + PRF membrane) may explain the difference of the results when compared to our PRF + BDX group.

There are no other studies with PRF and bone graft combinations where hard tissue parameters were evaluated using either re-entry or sounding methods. Studies by Bansal and Bharti (21), Agarwal et al (20) and Elgendy et al. (22) evaluated the changes in hard tissue levels only by the use of various radiographic methods. Therefore, it is not possible to compare their results with our sounding measurements.

Evaluation of regenerative treatment outcomes requires the assessment of hard tissue level changes. This may be done using clinical measurements however, it is important to reinforce the data with radiographic methods. Radiographic assessments are simple and atraumatic yet as effective when compared to re-entry and sounding methods (75). In our study, radiographies were taken using long cone paralleling technique and measurements were done from DSD to root apex using millimetric X-ray grids. The literature presents various studies conducted with a similar radiographic method (58). In our study, PRF + BDX and BDX groups presented 2.63 ± 0.98 (3) mm and 2.24 ± 1.03 (2) mm of Rad BG. For both treatment groups, Rad BG at 12 months was found to be statistically significant ($p < 0.01$) (Table 8). These results are in conjunction to the values obtained with sounding measurements, of which PRF + BDX and BDX groups revealed 2.74 ± 1 (3) mm and 2.10 ± 1.2 (2) mm of clinical bone gain, respectively. The difference in Rad BG between the two treatment groups was found to be statistically significant in favor of PRF + BDX group ($p < 0.05$) (Table 8).

Relevant literature provides 2 other studies with BDX treatment groups where similar radiographic assessments were reported (58, 87). Following a 12-month evaluation period, Scabbia and Trombelli (58) reported 4.4 mm PD reduction, 4.00 mm attachment gain and 3.1 mm Rad BG where baseline PD was reported to be 7.5 mm. When compared to our BDX treatment group, all parameters reported by Scabbia and Trombelli (58) are higher. This difference may be due to inclusion of IBDD ≥ 4 and higher baseline PD values in Scabbia and Trombelli's (58) study. Gupta et al. (87) conducted a study with similar inclusion criteria where BDX treatment group presented 3.27 mm of Rad BG. The higher outcome reported in this study may be explained by the inclusion of

only 2- and 3-wall defects as well as relatively short follow-up period of 6 months considering the slow resorption rate of BDX.

There is only one study where PRF + BDX combination was evaluated, however, no data on radiographic outcomes were reported (23). Elgendy et al. (22) used PRF membranes together with nanocrystalline HA grafting material in their test group and used radiographic examination for evaluating the treatment effects on bone. At 6 months, radiographic examination was repeated and results were compared to baseline in regards to the change in bone density. Following data were reported for the test group; bone density increased from 73.14 to 107.59. No data on Rad BG were reported. Bansal and Bharti (21) applied minced PRF pieces mixed with DFDBA into intrabony defects of their test group and results were evaluated at 6 months. They reported 2.13 mm of defect fill. In the study of Agarwal et al. (20), patients were either treated with PRF + DFDBA mixture (test) or DFDBA (control). In both groups, grafted defects were covered with a PRF membrane. At 12 months, test and control groups resulted in bone fill of 3.50 mm and 2.49 mm. Control group of this study is similar to our PRF + BDX group in terms of PRF application protocol where, bone fill was reported to be slightly lower (2.49 mm vs. 2.63 ± 0.98 (3) mm) compared to our results. These differences in the results might be explained by application of different graft materials.

In the literature, PRF has been shown to improve the regenerative outcomes in the treatment of periodontal intrabony defects when compared to OFD (26, 28-31). Furthermore, in two other studies, effects of different graft materials were compared to PRF and the authors reported both treatment options to be equally effective (24, 25). Lekovic et al. (23) demonstrated that the use of a graft material in addition to PRF improved the treatment outcomes. Studies by Elgendy et al. (22) and Bansal and Bharti (21) evaluated the use of PRF and bone graft combination and reported that combination therapy yields better results when compared to graft materials alone. Therefore, the literature supports the use of PRF in combination with a graft material. However, current literature does not contain any reports regarding the benefit of using PRF in addition to a graft material such as well-documented and widely used BDX.

Results obtained from our study demonstrated that the PRF + BDX combination yields significantly better results in regards to soft tissue parameters including, gingival recession, attachment gain, and hard tissue parameters consisting of bone gain and Rad BG compared to BDX alone over a 12-month follow-up period.

Healing after periodontal surgery and organization of the wound starts to take place almost immediately. Although remodelling/tissue maturation is a long process, new tissue formation is completed in a matter of 2-3 weeks following wound closure, and is key to success in regenerative periodontal treatment. Therefore it is suggested that, the biomaterials used during periodontal regenerative therapies should be aimed at improving healing outcomes during this early phase of wound healing (56). PRF biomaterial has been shown to release various growth factors and cytokines in the wound site for at least 7 days and doing so it has been shown to augment the early healing process (14, 139).

Statistically significant differences observed between PRF + BDX and BDX groups with regards to soft tissue parameters may be attributed to beneficial effects of PRF in promoting neoangiogenesis and remodelling of the gingival tissues (139). When compared to BDX group, statistically significant improvements in hard tissue parameters were observed favoring the PRF + BDX group. Using the PRF membrane is proposed to cover and protect the blood clot and/or graft material inside the defect area. On the other hand, improved soft tissue healing may result in periosteal coverage of the defect site with a resilient gingiva. This promoted periosteum coverage may function as a natural barrier between the soft tissue and hard tissue compartments, therefore may acts as a regenerative barrier thereby enhancing the regenerative outcomes (98).

In the light of this study, it can be concluded that the use of both PRF + BDX and BDX alone were successful in the treatment of periodontal intrabony defects and PRF + BDX combination has superior clinical and radiographic outcomes.

The evidence available in the literature for the clinical effects of PRF in the treatment of intrabony defects are limited. Further randomized controlled studies with various control groups, extended follow-up periods and high number of subjects are needed to investigate the advantages of PRF in the treatment of intrabony defects.

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