

Effect of PRGF on Bone Grafting of Intrabony Defects in Aggressive Periodontitis Patients – A Pilot Study

Rafik Dib, DDS, MSD, Pharm D[†], A. Archontia Palaiologou, DDS, † Pooja Maney, BDS, MPH, PhD[†]

Department of Periodontics
Louisiana State University Health Science Center
School of Dentistry
1100 Florida Avenue
New Orleans, LA 70119

Keywords: aggressive periodontitis, allografts, bone regeneration; periodontal attachment loss

Abbreviations: plasma rich in growth factors (PRGF), Aggressive periodontitis (AP), Freeze Dried Bone Allograft (FDBA), probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index (PI), gingival index (GI),

ABSTRACT (248/250 words)

Background: The use of plasma rich in growth factors (PRGF) is often incorporated in regenerative periodontal surgical procedures. However, the actual benefit of adding PRGF in intrabony defects in aggressive periodontitis patients remains undocumented. The purpose was to evaluate the potential of plasma rich in growth factors (PRGF) to enhance defect fill and attachment level gain of intrabony defects in aggressive periodontitis patients in combination with Freeze Dried Bone Allograft (FDBA).

Methods: Six patients with localized aggressive periodontitis with bilateral comparable intrabony defects (n=14) were treated. Sites were randomly assigned into one of two groups: control (FDBA) or test (FDBA/PRGF). The surgical procedure consisted of flap reflection, debridement of the defect, root planning, and placement of the bone graft in the defect, followed by flap closure. Clinical and radiographic parameters were assessed at baseline and 6 months after treatment.

Results: At baseline, there were no statistically significant differences between groups. At 6 months, both groups experienced improvement in terms of probing depth reduction, clinical attachment level (CAL) gain, clinical bone level gain and radiographic bone fill. However, no statistically significant difference was observed between the two groups. The test (PRGF/FDBA) and control group (FDBA) experienced a mean clinical

attachment level gain of 1.93mm and 2.21mm, respectively (P=0.36). The mean bone gain for the test group was 2.5mm, and 1.86mm for the control group (P=0.27).

Conclusion: Addition of PRGF on bone grafting of intrabony defects in aggressive periodontitis patients did not significantly enhance defect fill and attachment level gain.

INTRODUCTION

Aggressive periodontitis (AP) is a disease that is characterized by rapid attachment loss and bone destruction around teeth. AP has a strong familial tendency and is associated with immunologic defects and a distinct microbial flora. (1) It mainly targets healthy teenagers and young adults with the highest incidence among African Americans (2.6%) followed by Hispanic Americans (0.5% - 1.08%), whereas White Americans had the lowest prevalence of aggressive periodontitis (0.06% - 0.17%). (2, 3)

AP presents in two forms; localized and generalized. Localized AP usually has a circumpubertal onset (11-14 years of age), with periodontal damage (attachment and bone loss) to the permanent first molars and incisors. Generalized AP usually affects patients around 30-35 years of age, with generalized attachment and bone loss affecting at least three permanent teeth other than first molars and incisors [Lang, 1999 #4].

The overall goals of the treatment of AP are not different from those in patients with chronic periodontitis. A nonsurgical phase of scaling and root planing with systemic antibiotics takes place to alter or eliminate the microbial etiology and contributing risk factors. In addition, when indicated, surgical approach such as regenerative treatment of lost periodontal tissues may be attempted. (4, 5)

Periodontal regeneration is the process by which a lost or injured part of the periodontium (including the periodontal ligament, cementum and alveolar bone) is

reconstituted or reproduced. (6) AP may present with a pattern of vertical or angular bone loss defects, also known as a periodontal intrabony defects, which may respond well to regenerative procedures. (5)

Various grafting materials used for periodontal regeneration in patients diagnosed with AP have been well documented. Using Freeze-Dried Bone Allograft (FDBA) alone or with adjunctive systemic tetracycline showed improvement in periodontal parameters and defect bone fill (7, 8). A Combination of root biomodification, Decalcified Freeze-Dried Bone Allograft (DFDBA) and barrier membrane is also an effective therapy for periodontal intrabony defects in AP. (9) Alloplastic grafting materials have also been tested in a split-mouth design in combination with tetracycline where significant decreases in defect depth and pocket depth were detected. (10)

More recently, growth factors have been shown to also play an important role in tissue development and healing. Among the growth factors, plasma rich in growth factors (PRGF)* as described by Anitua et al has demonstrated the potential to influence bone regeneration. (11) In recent *in vitro* studies, PRGF demonstrated a stimulating effect on both alveolar osteoblasts and gingival fibroblasts , suggesting a possible beneficial effect in regeneration of periodontal tissues. (11, 12)

PRGF takes full advantage of concentrated platelets and stored growth factors by eliminating erythrocytes and leucocytes from the product. (13) The removal of leukocytes from the product also attempts to avoid the pro-inflammatory effects of the

* Endoret ®, BTI Biotechnology Institute, Vitoria, Spain.

proteases and acid hydrolysases.(14) PRGF releases growth factors and proteins that are involved in wound healing. These growth factors and proteins include fibrinogen, fibronectin, vitronectin, platelet derived growth factor, transforming growth factor β , vascular endothelial growth factor, insulin like growth factor, angiopoietin, platelet factor 4, and thrombospondin. (13)

The use of PRGF has been reported to enhance healing of extraction sockets by minimizing postoperative complications and stimulating the hard and soft tissue regeneration.(15) It has also been used in combination with xenografts as grafting for maxillary sinus grafting in humans.(16, 17) A case series has indicated a reduction of pocket depth and gain in attachment using platelet gel biotechnology in AP patients who had shown a refractory response to previous periodontal treatments.(18) However, a recent systematic review on autologous platelet concentrates was unable to consider the effect of PRGF in the treatment of intrabony defects due to the absence of randomized clinical studies [Saurav 2016 #20]. To our knowledge, the effectiveness of PRGF in patients diagnosed with AP is still unknown. The objective of this study is to evaluate the additive effect of PRGF with FDBA[†] in the treatment of intrabony defects in aggressive periodontitis. Our hypothesis is that the addition of PRGF to FDBA will enhance defect fill and attachment level gain.

MATERIALS AND METHODS

[†] Creos allo.gain cortico-cancellous (0.25-1.00 mm) – Nobel Biocare

Following LSUNO-IRB approval (IRB# 9004), participants were recruited among patients referred to the Department of Periodontology at the Louisiana State University for treatment of aggressive periodontitis (AP), diagnosed according to the criteria established at the 1999 international workshop for the classification of periodontal diseases and conditions. (19) 47 Patients were screened, 8 were eligible, consented and were enrolled. All 8 were African American females, aged 14 to 40 years old.

Inclusion criteria were: 1) male or female between 13-40 years of age; 2) must be diagnosed with localized or generalized aggressive periodontitis with bilateral intrabony defects that require grafting, ideally with symmetrical walls and at least 3mm deep around the tooth/teeth for which periodontal flap surgery with bone grafting is indicated (one, two and three wall intrabony defects); 3) must be of sufficiently good health to undergo routine dental treatment including gingival surgery; 4) must demonstrate acceptable oral hygiene (Modified O'Leary plaque score of $\geq 80\%$) prior to surgical therapy

Exclusion criteria were: 1) have any requirements for antibiotic premedication for heart murmurs, artificial joints or any other condition; 2) have a history of significant heart, stomach, liver, kidney, blood, immune system or other organ impairment or systemic disease that would preclude their undergoing the proposed treatment; 3) are taking drugs known to interfere with wound healing (e.g. corticosteroids, chemotherapeutic anti-cancer drugs, immune modulators etc.) or have received such drugs within 4 weeks of treatment. 4) have taken systemic antibiotics or any investigational drugs anytime in the previous month; 5) have other dental conditions likely to require treatment, necessitating exit from the study e.g. decision to extract a

tooth that was considered for the study due to failure of root canal therapy or extensive tooth decay beyond the limit that is considered successfully restorable; 6) have had periodontal surgery on the involved teeth within the last 6 months; 7) cannot comply with the extra treatment visits and follow-up visits for up to 6 months; 8) lack of appropriate periodontal bony defects; 9) heavy alcohol intake or use of illicit drugs (The Substance Abuse and Mental Health Services Administration (SAMHSA), defines heavy drinking as drinking 5 or more drinks on the same occasion on each of 5 or more days in the past 30 days. SAMHSA defines binge drinking as drinking 5 or more alcoholic drinks on the same occasion on at least 1 day in the past 30 days); 10) female subjects must not be pregnant or nursing for study surgeries. If they become pregnant during the 2 year follow up period, only clinical measurements would be made, but no radiographs would be taken.

Study Design

Defects were grafted with either FDBA/PRGF or FDBA alone utilizing a randomized split mouth design (Figure 1). The randomization was performed by a coin toss.

Clinical Parameters

Clinical parameters were assessed using a stent made of clear suck-down A+ plastic material[‡] trimmed to the coronal 2/3 and a manual UNC-15 probe.

Defect-specific assessments were made at the deepest point of the defect (base of the defect (BD)). Interproximal embrasures of the stent were used as fixed reference points

[‡] Essix A+® Plastic

to aid in alignment (**figure 2b**). All measurements were completed by the same clinician (RD).

For each patient, the following parameters were assessed on two sites per tooth (mesial buccal and mesial lingual) at baseline and 6 months reentry: 1) Probing depth (PD); 2) Recession (REC); 3) Bleeding on probing (BOP); 4) plaque index (PI);(20) 5) gingival index (GI).(20) The parameters recorded during surgery are 1) stent to clinical base of defect 2) CEJ to clinical base of defect.

Radiographic Parameters

Standardized periapical radiographs were obtained at baseline and at 6 months reentry using a radiographic stent. Bite-record was taken during baseline with Thixotropic Vinyl Polysiloxane[§] to standardized the sensor position intra-orally. The distance from the radiographic CEJ to the base of the defect at baseline and at 6 months of reentry were measured using a digital radiographic software^{**}. Radiographic bone fill was assessed by calculating their mean difference.

Initial examination:

A complete medical and dental history was taken. Subjects received a routine periodontal examination including charting of probing depths, attachment level and gingival recession at 6 sites per tooth. Mobility, furcation involvement, gingival index

[§] Blu-Mousse® - Parkell Inc

^{**} Schick CDR DICOM 5 Digital-X-ray-Software by Dentsply Sirona Imaging

and plaque index were also assessed. Full mouth series of radiographs and intraoral photographs were taken.

Initial therapy:

All subjects received scaling and root planing of teeth with PD \geq 4mm. Antibiotics (Amoxicillin 500mg and Metronidazole 250mg three times a day for 10 days) were prescribed. Detailed oral hygiene instructions were given; brushing twice daily using a soft bristle tooth brush using modified Bass technique and floss once daily.(21) After 4-6 weeks, re-evaluation of initial therapy was completed. Standardized measurements were recorded around the teeth that require grafting of defects using a custom-made stent.

Surgical therapy:

Preparation of the autologous PRGF: 10-20 mL of peripheral blood were drawn using venipuncture from the subject's arm. PRGF was prepared using the protocol described by Anitua E [Anitua, 2014 #24]. Briefly, this consists of centrifuging the blood sample for 8 minutes at ambient temperature. After centrifugation, the blood will be separated into 3 phases (erythrocyte cells at the bottom, above a thin layer of leukocytes (buffy coat), and plasma at the top). The plasma layer is divided into two fraction; fraction 1 (F1) and fraction 2 (F2). F2 of PRGF is the 2mL of plasma just above the buffy coat. It is the fraction that is richest in platelets between 2-3 times higher than that of peripheral blood.(13) Calcium chloride (PRGF-Activator) was added to the carefully pipetted out F2, according to the manufacturer's instructions. The graft material (FDBA) was mixed

into the serum right after activation. The mixture is then placed in a 37°C furnace^{††} around 10 minutes for a fibrin clot formation. The resulting gel-like substance containing the graft was then placed into the periodontal intrabony defect.

Surgical treatment of intrabony defects (Figure 2.): Intrasulcular incisions were made including at least one tooth on either side of the tooth to be treated and full thickness gingival flaps was elevated. The surgical sites were thoroughly debrided to remove granulation tissue and calculus deposits using hand and ultrasonic instruments. The defects were measured using a custom-made stent (St-BD) and the number of remaining walls were determined. Defects were grafted randomly with the materials under study utilizing a split mouth design. One side treated with PRGF+FDDBA and the other side was treated with FDDBA alone. Flaps were sutured using 4-0 Vicryl interrupted sutures attempting to gain primary closure. Post-operative instructions were given. Post-operative examinations were done to monitor healing of the surgical sites at 1 week, 2 weeks and 3 months. All patients were prescribed amoxicillin 500mg three times daily for 7 days, ibuprofen 600mg every 6-8 hours and chlorhexidine gluconate 0.12% rinse twice daily for 2 weeks. Sutures were removed 1 week postoperatively. Recall appointment were scheduled 2 weeks post-operatively and 3 months for supragingival debridement and reinforcement of oral hygiene.

Evaluation of treatment:

^{††} Plasmaterm H. - BTI Biotechnology Institute

Clinical and radiographic data were recorded at 6 months following surgery.

Clinical parameters were re-assessed by a re-entry procedure at 6 months, utilizing a mini-flap that just reflected the interproximal buccal and lingual gingival papilla to visualize the grafted site and to take measurements using the custom-made stent (Figure 3). Probing depths, attachment level, surgical height of crest of alveolar bone, and base of defect were measured. Standardized radiographs of the study sites were taken at baseline and 6 months re-entry (Figure 4).

Statistical Analysis

Pre-treatment measurements were compared to the measurements recorded at 6 and 12 months. A one-sample t-test was used to determine if there is a significant clinical attachment gain and defect fill in intrabony periodontal defects treated with PRGF/FBDA at both time points. Significance was set at $P=0.05$.

RESULTS

Of 47 screened patients, 8 eligible and consenting participants were accepted. 2/8 patients were excluded due to shape of defect not meeting criteria at surgery (intrabony defect $<3\text{mm}$). Thus, 6 patients completed the study with bilateral defects ($n=14$). 1 out of the 6 patients had two bilateral defects, one in each arch. All defects were located on the mesial of maxillary or mandibular first molars. Patient's ages ranged from 16 to 32 years, with a mean age of 19.5 year. They were all African American, females, nonsmokers. Patient demographics and defect assignment are reported on Table 1.

Baseline and 6 months parameters are reported in Table 2. At baseline, there were no statistically significant differences between control and test groups for any of the recorded parameters.

The changes in clinical parameters, comparing baseline to 6 months values, are reported in (Figure 5a-f). At 6 months, both groups experienced improvements in terms of probing depth reduction, clinical attachment level (CAL) gain, and bone level gain. In addition, both resulted in similar level of radiographic bone fill.

The test (PRGF/FDBA) and control group (FDBA) experienced a mean clinical attachment level gain of 1.93mm and 2.21mm, respectively ($P=0.36$) (Figure 5a). Mean Probing depth decrease was 1.93mm for the test group and a 1.93mm for the control group ($P=0.36$) (Figure 5b). Mean bone gain for the test group was 2.5mm, and a 1.86mm for the control group ($P=0.27$) (Figure 5c). Clinical parameters such as BOP, PI, GI had all improved from baseline to 6 month. Mean BOP reduction for the test group was 0.14, and a 0.21 for the control group ($P=0.3$) (Figure 5d). Mean GI reduction of 0.36 for the test group and a 0.5 reduction for the control group ($P=0.18$) (Figure 5e). Mean PI reduction of 0.29 for the test group and 0.14 for the control group ($P=0.09$) (Figure 5f).

CONCLUSION

In summary, all clinical parameters improved within the control and test groups at 6 months as compared to baseline measurement. No statistically significant difference was found between the two groups. This study demonstrated that the addition of plasma rich in growth factors (PRGF) in the treatment of intrabony defects did not enhance

defect fill and clinical attachment level gain in patients with aggressive periodontitis (AP). Further long-term and large-sample size evaluation may be required to further confirm this pilot study's results.

DISCUSSION

The present study evaluated the additive effect of PRGF with FDBA in the treatment of intrabony defects in aggressive periodontitis patients. To avoid the effect of natural variations between different individuals, gender, race, smoking status and location of defects were matched throughout the study. 14 defects in 6 female, African American patients, non-smokers, with bilateral mesial of first molar comparable defects were randomly assigned to either the test or the control group.

Patient's compliance with dental appointment was a challenge for the recruited patient's population. Multiple failed appointments have occurred in the non-surgical phase. According to Modin et al, they reported that the patient group with aggressive periodontitis interrupted the periodontal treatment significantly more often (46%) irrespective of background factors and risk factors, compared to patients with chronic periodontitis (34%).(22) In addition, the non-compliant patients had significantly deeper periodontal pockets at baseline as well as significantly more sites with bleeding at probing ($p < .01$). (22) To improve compliance, educating and increasing awareness to both patients and parents took a primary role. (23) In this study, verbal and written instructions was provided to both the patients and their parents. The goal of the provided instructions was to increase awareness of the severity of the disease and stress the necessity to continue the surgical phase and the 6 months re-entry visit.

A similar study design by Ravi et al. was recently published to evaluate the effect

of PRGF with GTR as well as GTR alone in the treatment of intrabony defects in chronic periodontitis patients.(24) At re-entry, all clinical parameters showed improvement but, intergroup comparison did not show a statistical difference.(24) Thus, there was no additional benefit of PRGF when used along with GTR in treatment of chronic periodontitis patients.(24) Our findings are in agreement with the findings of that study.

There were some limitations to our study. Since it is a pilot study, the sample size was small. Only 6 patients completed the study for a total of 14 defects using the split mouth design.

Effort was made to identify comparable or symmetrical bilateral defects in the same participant. However, due to the complex anatomy of each defect, it was difficult to match the osseous defects in the test and control groups in all participants. The randomization and the selection of only mesial first molar defect may have helped to control this variability.

Furcation involvement was observed in more than half of the defects. Furcation involvement in molar teeth render a less favorable prognosis.(25) In addition, evidence shows that grafting in a furcation area results in about 50% improvement for grade II furcation.(26) Of these, 20% were completely filled and 33% improved from grade II to grade I.(26)

Standardized radiographs taking presented additional challenges. In some cases, the measurements from the CEJ to the base of the defect was very difficult, due to the CEJs being difficult to identify or new bone levels were not well defined. Even though radiographs were standardized, data collection varied widely between examiners. For this reason, radiographic analysis was excluded from the results of this

study.

Finally, defective restorations and margins were found in several molars where old restorations should have been replaced prior to the surgical procedure for better surgical prognosis.

The use of PRGF has several advantages in clinical practice in comparison with other regenerative materials, as it is easier to handle in combination with grafting materials, results in accelerated post-operative soft tissue healing and causes less patient discomfort.(27) However, there is also a lack of evidence identifying the effect of PRGF on postoperative symptoms like pain and swelling.

As the existing study exhibits methodological limitations, future studies with a long-term evaluation and a larger sample size are necessary to confirm the clinical stability of this therapeutic approach in addition to a larger sample size. There is a need for further randomized controlled clinical trials to provide accurate information on the effect of PRGF for periodontal intrabony defect treatment in patients with aggressive periodontitis.

Table 1.

Demographics of study population

research code	Age	Race	Gender	FDBA	FDBA+PRGF
X1	14	AA	F	19,14	30,3
X2	15	AA	F	30	19
X3	16	AA	F	19	30
X4	40	AA	F	14	3
X5	18	AA	F	19	30
X6	14	AA	F	14	3

Table 2.

Mean defect characteristic at baseline.

	FDBA (n=7)	PRGF/FDBA (n=7)	P value
PD	8.07	8.64	0.2
REC	0	0	n/a
BOP	0.64	0.64	0.55
GI	0	0	n/a
PI	0	0	n/a
St- BD	11.93	12.64	0.2

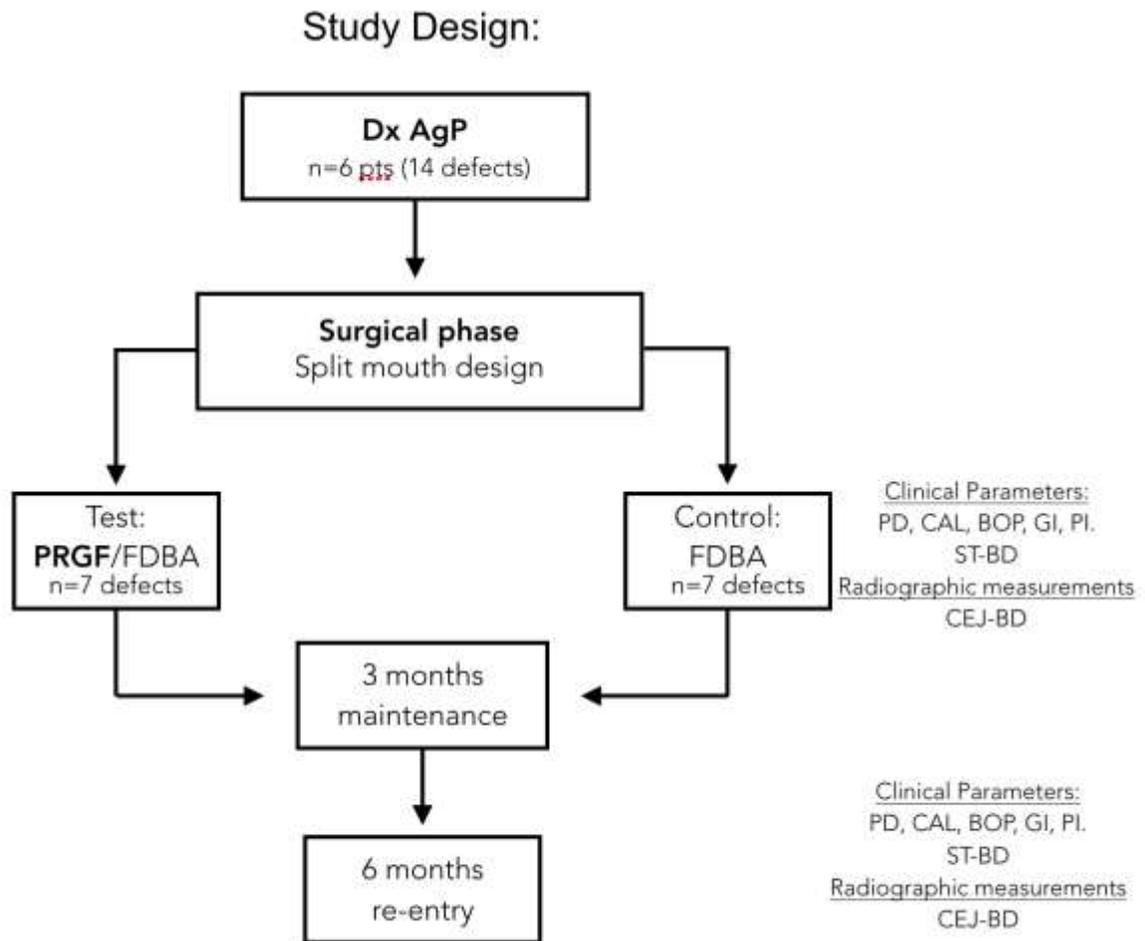


Figure 1. Study Design

Figure 2. Surgical protocol



Figure 2a. Full thickness flap reflected, degranulation of the defect.



Figure 2b. Clinical parameters were assessed using a stent made of clear suck-down material trimmed to the coronal 2/3 and a manual UNC-15 probe.



Figure 2c. Defect grafted with either Test (PRGF/FDBA) or control (FDBA)



Figure 2d. Primary closure with 3 interrupted 4-0 sutures

Figure 3. 6 months re-entry



Figure 3a. Mini-flap reflection on buccal and lingual



Figure 3b. Standardized measurements



Figure 3c. flap closure with 1 interrupted 4-0 Chromic gut suture

Figure 4. Baseline and 6 months re-entry measurement



Figure 4a. Baseline measurements $at:BD = 11mm$ presence of a mesial radiographic defect



Figure 4b. 6 months re-entry $at:BD = 9mm$ - resulting a 2mm of bone fill presence of a mesial radiographic defect fill

Figure 5a

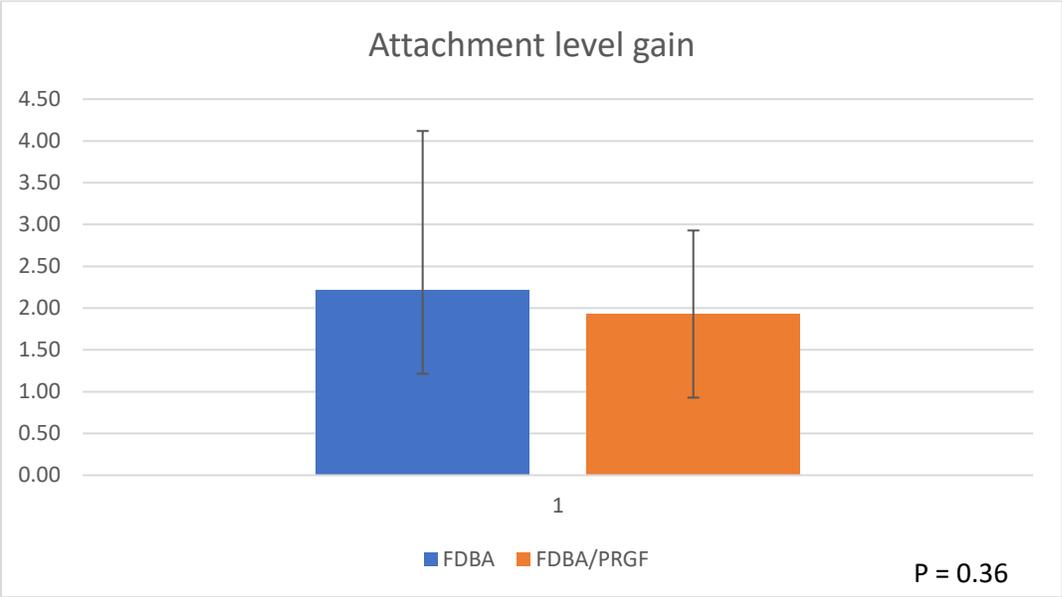


Figure 5b

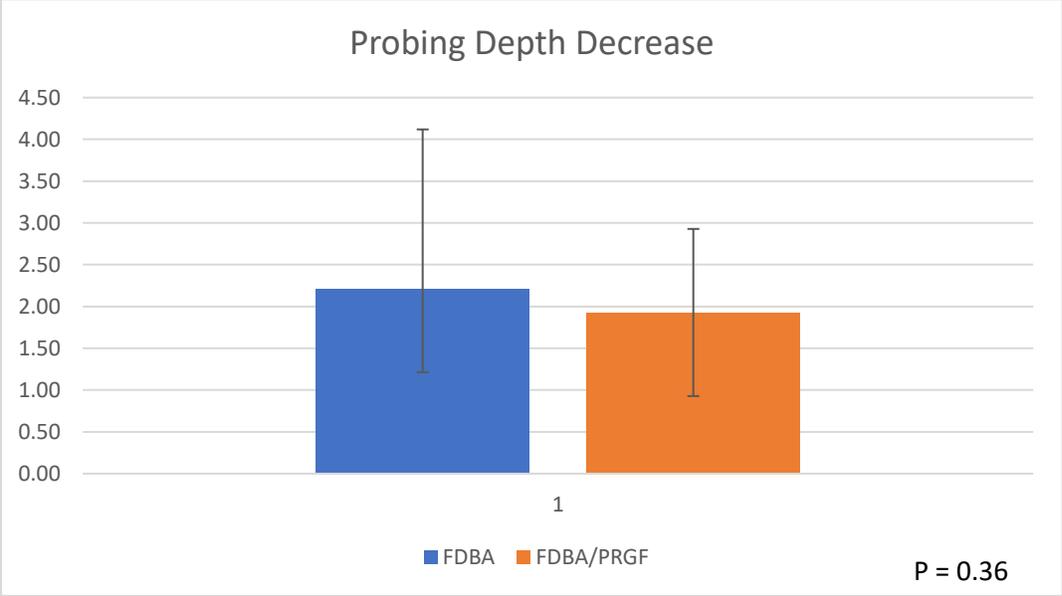


Figure 5c

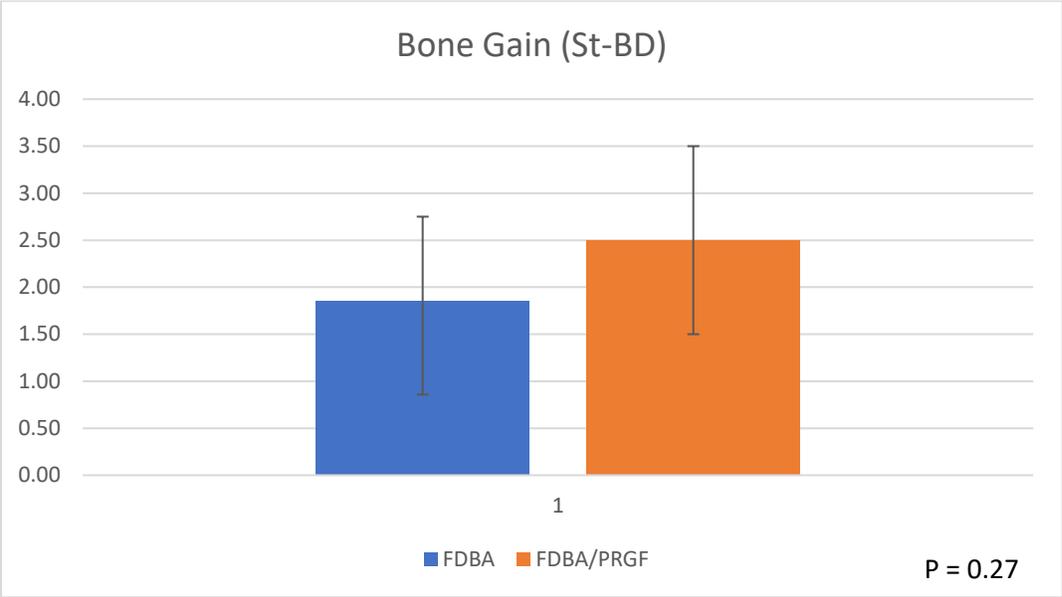


Figure 5d

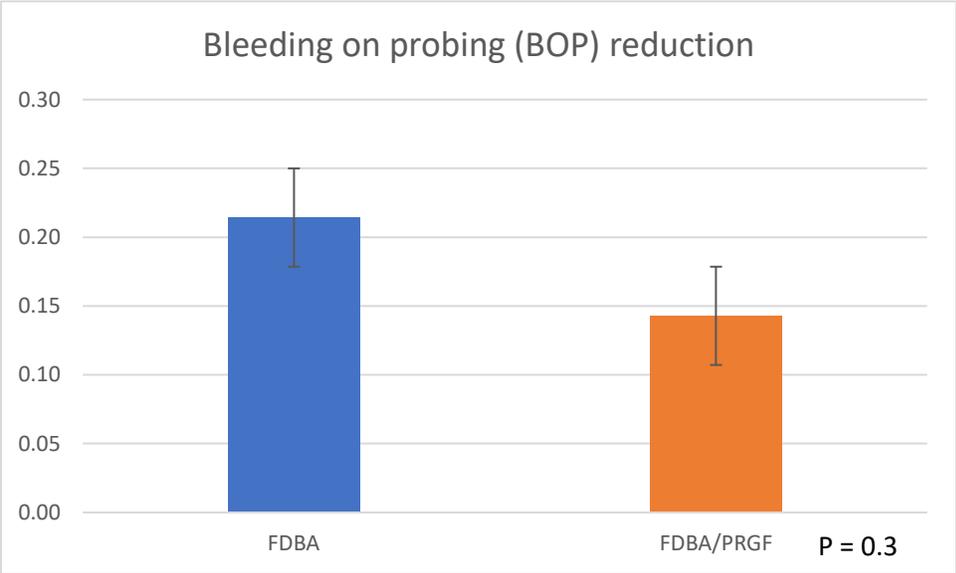


Figure 5e

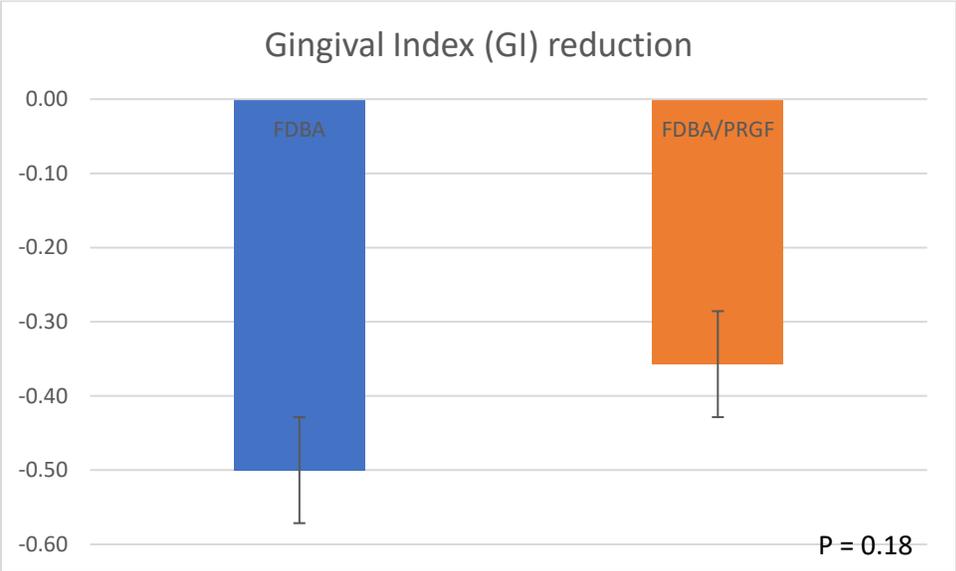
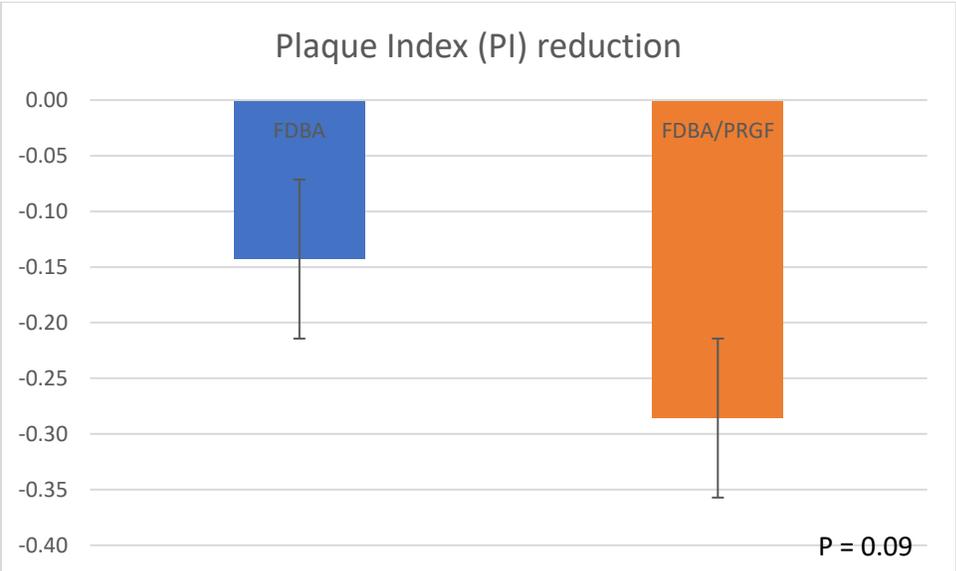


Figure 5f



1. Califano JV, Research S, Therapy Committee American Academy of P. Position paper: periodontal diseases of children and adolescents. *J Periodontol.* 2003;74(11):1696-704.
2. Albandar JM, Brown LJ, Loe H. Clinical features of early-onset periodontitis. *J Am Dent Assoc.* 1997;128(10):1393-9.
3. Loe H, Brown LJ. Early onset periodontitis in the United States of America. *J Periodontol.* 1991;62(10):608-16.
4. Parameter on aggressive periodontitis. American Academy of Periodontology. *J Periodontol.* 2000;71(5 Suppl):867-9.
5. Teughels W, Dhondt R, Dekeyser C, Quirynen M. Treatment of aggressive periodontitis. *Periodontol 2000.* 2014;65(1):107-33.
6. Lang NP. Focus on intrabony defects--conservative therapy. *Periodontol 2000.* 2000;22:51-8.
7. Mabry TW, Yukna RA, Sepe WW. Freeze-dried bone allografts combined with tetracycline in the treatment of juvenile periodontitis. *J Periodontol.* 1985;56(2):74-81.
8. Yukna RA, Sepe WW. Clinical evaluation of localized periodontosis defects treated with freeze-dried bone allografts combined with local and systemic tetracyclines. *Int J Periodontics Restorative Dent.* 1982;2(5):8-21.
9. Sant'Ana AC, Passanezi E, Todescan SM, de Rezende ML, Gregghi SL, Ribeiro MG. A combined regenerative approach for the treatment of aggressive periodontitis: long-term follow-up of a familial case. *Int J Periodontics Restorative Dent.* 2009;29(1):69-79.
10. Evans GH, Yukna RA, Sepe WW, Mabry TW, Mayer ET. Effect of various graft materials with tetracycline in localized juvenile periodontitis. *J Periodontol.* 1989;60(9):491-7.
11. Anitua E, Tejero R, Zalduendo MM, Orive G. Plasma rich in growth factors promotes bone tissue regeneration by stimulating proliferation, migration, and autocrine secretion in primary human osteoblasts. *J Periodontol.* 2013;84(8):1180-90.
12. Anitua E, Troya M, Orive G. Plasma rich in growth factors promote gingival tissue regeneration by stimulating fibroblast proliferation and migration and by blocking transforming growth factor-beta1-induced myodifferentiation. *J Periodontol.* 2012;83(8):1028-37.
13. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost.* 2004;91(1):4-15.
14. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res.* 2007;25(2):230-40.
15. Anitua E, Murias-Freijo A, Alkhraisat MH, Orive G. Clinical, radiographical, and histological outcomes of plasma rich in growth factors in extraction socket: a randomized controlled clinical trial. *Clin Oral Investig.* 2015;19(3):589-600.
16. Del Fabbro M, Bortolin M, Taschieri S, Weinstein RL. Effect of autologous growth factors in maxillary sinus augmentation: a systematic review. *Clin Implant Dent Relat Res.* 2013;15(2):205-16.
17. Khouly I, Pardinias Lopez S, Aliaga I, Froum SJ. Long-Term Implant Survival After 100 Maxillary Sinus Augmentations Using Plasma Rich in Growth Factors. *Implant Dent.* 2017;26(2):199-208.
18. Mauro S, Orlando L, Panzoni R, Orlando PF. Platelet gel biotechnology applied to regenerative surgery of intrabony defects in patients with refractory generalized aggressive periodontitis. Case report. *Minerva Stomatol.* 2003;52(7-8):401-12.

19. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol.* 1999;4(1):1-6.
20. Loe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol.* 1967;38(6):Suppl:610-6.
21. Poyato-Ferrera M, Segura-Egea JJ, Bullon-Fernandez P. Comparison of modified Bass technique with normal toothbrushing practices for efficacy in supragingival plaque removal. *Int J Dent Hyg.* 2003;1(2):110-4.
22. Modin C, Abadji D, Adler L, Jansson L. Treatment compliance in patients with aggressive periodontitis - a retrospective case-control study. *Acta Odontol Scand.* 2017;75(2):94-9.
23. Wilson TG, Jr., Hale S, Temple R. The results of efforts to improve compliance with supportive periodontal treatment in a private practice. *J Periodontol.* 1993;64(4):311-4.
24. Ravi S, Malaiappan S, Varghese S, Jayakumar ND, Prakasam G. Additive Effect of Plasma Rich in Growth Factors With Guided Tissue Regeneration in Treatment of Intrabony Defects in Patients With Chronic Periodontitis: A Split-Mouth Randomized Controlled Clinical Trial. *J Periodontol.* 2017;88(9):839-45.
25. Miller PD, Jr., McEntire ML, Marlow NM, Gellin RG. An evidenced-based scoring index to determine the periodontal prognosis on molars. *J Periodontol.* 2014;85(2):214-25.
26. Evans GH, Yukna RA, Gardiner DL, Cambre KM. Frequency of furcation closure with regenerative periodontal therapy. *J West Soc Periodontol Periodontal Abstr.* 1996;44(4):101-9.
27. Mozzati M, Martinasso G, Pol R, Polastri C, Cristiano A, Muzio G, et al. The impact of plasma rich in growth factors on clinical and biological factors involved in healing processes after third molar extraction. *J Biomed Mater Res A.* 2010;95(3):741-6.