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ORIGINAL ARTICLE

Demineralized freeze-dried bone allograft and platelet-rich plasma vs platelet-rich plasma alone in infrabony defects: a clinical and radiographic evaluation

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Abstract The objective of this work is to compare the clinical and radiographic outcomes of demineralized freeze-dried bone allograft (DFDBA)/platelet-rich plasma (PRP) combination with PRP alone for the treatment of infrabony defects 18 months after surgery and to examine the influence of radiographic defect angle on the clinical and radiographic outcomes. Twenty-eight infrabony defects were treated with DFDBA/PRP combination or PRP alone. Clinical parameters and radiographic measurements were compared at baseline and 18 months. Interquartile range was performed to classify the defect angles. Mann–Whitney, Wilcoxon test, and Pearson correlation were used to analyze the data. The DFDBA/PRP combination exhibited more favorable gains in both clinical and radiographic parameters than PRP alone group ($p<0.05$). A correlation existed between defect angle, defect depth, and clinical/radiographic outcomes for the defects treated with DFDBA/PRP. The narrow defects presented more favorable clinical attachment level values (CAL) gain, probing pocket depth (PPD) reduction and defect resolution than wide defects in the combination group ($p<0.05$). The influence of baseline defect angle was not significant in the PRP-alone group ($p>0.05$). The results indicate that DFDBA/PRP combination is more effective than PRP alone for the treatment of infrabony defects, and the amount of CAL gain, PPD reduction, and bone fill increases when the infrabony defect is narrow and deep before DFDBA/PRP combination treatment.

Keywords Demineralized freeze-dried bone allograft · Platelet-rich plasma · Infrabony defects · Defect angle · Defect depth

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Introduction

Demineralized freeze-dried bone allograft (DFDBA), which is shown to be both osteoconductive and osteoinductive, has been used alone and in combination with other treatment modalities for periodontal therapy for the last three decades [29, 37]. The observations from histological studies performed on humans and data from controlled clinical trials have shown that some of the available grafting procedures may result in healing that can be defined as “periodontal regeneration.” However, complete and predictable reconstruction of periodontal tissues is still difficult to obtain [3, 44].

The use of polypeptide growth factors (PGFs) in periodontal regeneration has recently attracted the attention of periodontal researchers. Despite their potential usefulness, animal-derived or genetically engineered PGFs are still not available for routine use in practice since their safety and effectiveness have not been completely confirmed [5]. A number of growth factors are sequestered in platelets including platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ), and insulin-like growth factor-I [1, 26]. A convenient approach to obtain autologous PDGF and TGFβ is the use of platelet-rich plasma (PRP) [26]. Early studies have focused on PRP application to bone graft material, showing that it leads to earlier bone regeneration and soft tissue healing, as well as greater density of mature trabecular bone [27]. Several studies using histological techniques suggest that PRP preparations may enhance local bone formation [13, 21, 27], while others have not been able to confirm these findings [1, 14, 15, 20].

In general, approximately 60–65% defect fill is expected after the use of a bone grafting material [17]. Although an improvement occurs, a residual defect usually exists. This has led researchers to investigate if a combination of regenerative therapies would promote maximum resolution of the defects. Since then, bone replacement grafting has been combined with a synthetic cell-binding peptide (P-15), guided tissue regeneration (GTR), enamel matrix derivative (EMD), and PRP [5, 24–26, 32, 41, 43, 44].

Except for different materials, infrabony defect characteristics were also shown to influence periodontal healing in earlier studies [9, 10, 12, 33]. It was reported that variability in clinical outcome may reflect the differences in defect characteristics, including preoperative attachment level, probing depth, infrabony wall components, defect depth, and defect angle [23]. In a recent study, Klein et al. [22] reported that narrow and deep infrabony defects respond radiographically and, to some extent, clinically more favorable to GTR therapy than wide and shallow defects. Using EMD for the treatment of infrabony defects, Tsitoura et al. [42] also observed an association between baseline radiographic defect angle and CAL gain at 1 year. However, there is no published data regarding the combined used of PRP with biomaterials (DFDBA) and the influence of defect characteristics on the clinical outcomes of this combination.

Therefore, the aim of the present study was to compare the clinical and radiographic outcomes of combined DFDBA/PRP and PRP alone for the treatment of infrabony defects 18 months after surgery, with a secondary aim to evaluate the influence of baseline defect angle on the treatment outcomes.

Materials and methods

Study design

The study was performed on patients who were referred to the Department of Periodontology, Faculty of Dentistry, Ege University for treatment of advanced chronic periodontitis. A 6-month time limit was determined for the selection of patients and infrabony defects. It was decided by toss-up that the patients with infrabony defects who applied to the Periodontology clinic in the first 3 months would have DBDFA + PRP treatment, while the patients in the last 3 months would have PRP therapy alone. Inclusion criteria were also defined for the selection of patients and infrabony defects: healthy adults between 18 and 60 years of age; presence of generalized advanced periodontal tissue destruction and presence of at least one deep infrabony defect as defined by X-rays; probing pocket depth and attachment loss ≥6 mm; one to three osseous walls; and absence of furcation involvement.

A total of 22 patients (13 women and 9 men, age range 26–54 years) who fitted the selection criteria applied to the Periodontology clinic in 6 months. Fourteen patients with 16 infrabony defects (eight women, six men, age range 26–48, two smokers), who applied in the first 3 months, received DFDBA + PRP combination treatment. The PRP-alone therapy group included eight patients with 12 infrabony defects (five women, three men, age range 28–54, one smoker). Before surgery, all subjects received nonsurgical periodontal treatment including subgingival scaling and root planning. Oral hygiene instructions were given and compliance was recommended to obtain an full-mouth plate score (FMPS) and an full-mouth bleeding score (FMBS) of at least ≤20%, a plaque index (PI)=0 and a gingival index (GI)=0 at the level of the surgical site by the day of surgery. To avoid a bias in statistical analyses, a comparison was performed between the two groups regarding preoperative clinical parameters before periodontal surgery. No statistically significant differences were found between the mean preoperative probing pocket depth (PPD; $p=0.23$) and mean clinical attachment level values (CAL; $p=0.24$) between the two treatment groups. A written informed consent was obtained for all the participants and the University Institutional Review Board approved the study design.

Surgical procedure

Platelet-rich plasma preparation

Ten milliliters of blood was drawn from each patient by venipuncture of the antecubital vein. Blood was collected into glass tubes containing 10% trisodium citrate solution as an anticoagulant. The glass tubes containing the blood were centrifuged at 5,600 rpm. for 6 min, which resulted in the separation of three basic fractions. Platelet-poor plasma (PPP) was on top of the preparation, PRP in the middle, and the red blood cell (RBC) fraction at the bottom. Two milliliters of the top layer corresponding to PPP were aspirated with a Pasteur pipette and discarded. The PRP was collected in conjunction with the top 1–2 mm of the RBC fraction, as the latter is also rich in newly synthesized platelets.

All clinical measurements were performed by the same examiner (T.I.) at study baseline and 18 months after treatment with a 15-mm probe to the nearest 0.5 mm. (PCP-

UNC 15, Hu-Friedy, Chicago, IL). A sulcular incision with full thickness flap was employed during surgeries. Care was exercised to preserve as much interproximal soft tissue as possible. A through debridement of the lesion including root planning was performed. PRP was mixed with DFDBA (Dembone, Pacific Coast Tissue Bank, Los Angeles, CA) and placed until it is filled with a periosteal elevator into the osseous defect during the surgery procedures of the combination group. The other group received only PRP for the treatment of infrabony defects. Dressing (Peripac, Dentsply DeTrey GmbH, Germany), and silk sutures were removed 1 week postoperatively. Surgical wounds were gently cleansed with 0.12% chlorhexidine gluconate on a cotton swab. Patients were instructed to rinse with 0.12% chlorhexidine gluconate during the second postoperative week.

Radiographic evaluation

Standardized radiographic examination was carried out preoperatively and 18 months postoperative. Before the exposure, individual occlusal acrylic stents were prepared for each patient. Periapical radiographs were taken (Ektaspeed, Eastman Kodak, Rochester, NY, USA) using the parallel technique (70 kVp, 10 mAs, Trophy ETX, Trophy Radiologie, Vincennes, France) and film holders (RWT, KKD, Ellwangen/Jagst, Germany). The films were developed in an automatic film processor (XR 24; Dürr-Dental, Bietigheim, Germany). All radiographs were digitized using a scanner with a transparency module (Hewlett-Packard Scanjet XPA 7400c, Avision, China) to a resolution of 256 pixels with 8 bits of gray-level resolution per pixel and saved as TIFF files. The images were then transferred to a software program that produces geometric standardization (Emago/Advanced, version 3.1, Oral Diagnostic Systems, Amsterdam, Netherlands). The preoperative image of each defect was used as the reference, and each postoperative image was reconstructed according to its reference by selecting four points on the preoperative image. The following measurements were performed on both preoperative and postoperative radiographs (Fig. 1):

- Distance between cementoenamel junction and alveolar crest (CEJ-AC)
- Distance between cemento-enamel junction and base of the defect (CEJ-BD)
- Distance between alveolar crest and base of the defect (AC-BD)

If the CEJ was destroyed by a restorative treatment, the margin of the restoration was taken as a landmark. BD was defined as the most coronal point where the periodontal ligament space showed a continuous width. If no periodontal ligament space could be identified, the point where the projection of the AC crossed the root surface was taken as a landmark [2]. If both structures could be identified at one defect, the point defined by the periodontal ligament was used as BD and the crossing of the silhouette of the alveolar crest with the root surface was defined as AC. If several bony contours could be identified, the most apical one that crossed the root was defined as the BD and the most coronal one as AC [11].

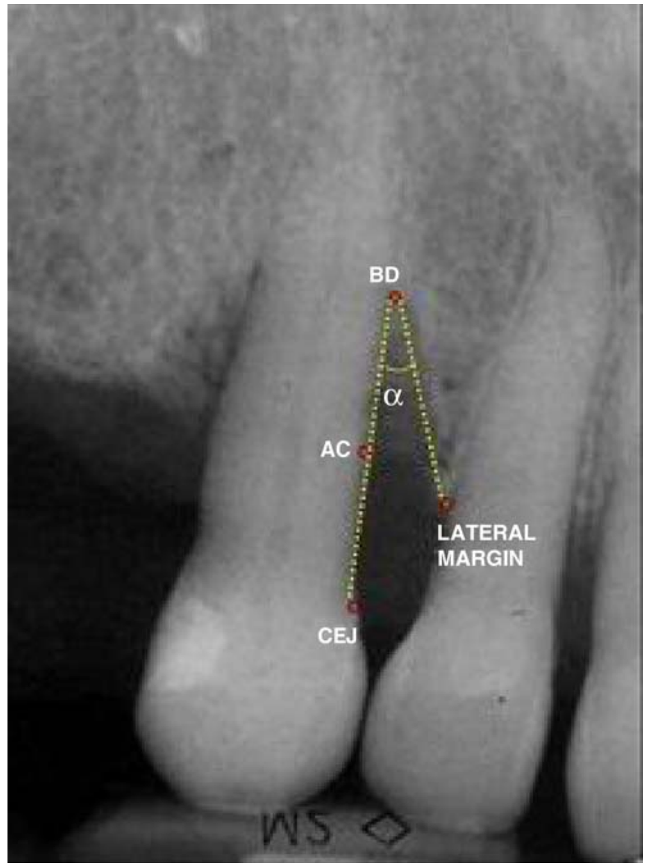

Fig. 1 The anatomical landmarks and defect angle (α)

The defect width was measured from the lateral margin of the infrabony defect to the landmark AC on the root surface. Further using the function angle, one side of an angle was drawn from the CEJ to the BD and the other from BD to the lateral margin of the infrabony defect. The program calculated the angle between these two lines. The measurements, which were recorded as millimeters and degrees, were repeated three times by an investigator who had not participated in the clinical treatment.

Statistical analyses

SPSS 3.0 (SPSS, Chicago, IL) was used to analyze the data. The comparison of baseline clinical and radiographic measurements and the comparison of the changes from baseline to 18 months between the two treatment groups were performed with Mann–Whitney test. Comparison of measurements between baseline and 18 months within each group was carried out using nonparametric Wilcoxon test. The correlation matrix between various clinical and radiographic measurements within the two groups was evaluated with Pearson correlation coefficient. The radiographic defect angle was categorized as narrow, intermediate, and wide, based on interquartile ranges, which were performed separately for the two treatment groups. The defect angles that were ≤25 were defined as narrow, while angles ≥30 were defined as wide for the PRP-alone therapy group. For the DFDBA + PRP treatment group, defect angles that were ≤18 were defined as narrow and angles ≥33 were defined as wide based on interquartile ranges. Mann–Whitney test was used to compare the clinical and radiographic changes between initially narrow and initially deep infrabony defects within each treatment group. There was no need for power analyses since the study was connected to a deadline. However, a power calculation ($1-\beta$) was performed when there was a statistically nonsignificant difference regarding clinical and radiographic parameters. A p value less than 0.05 was accepted to identify a statistically significant difference in all analyses. The differences between smokers and nonsmokers were negligible. Since only three of the patients were smokers, these data was not analyzed statistically.

Results

All sites healed uneventfully with no clinically detectable or subjectively reported side effects. Baseline and 18 months post-surgery, a total of 56 standardized radiographs were obtained from 28 infrabony defects in 22 patients. Two patients contributed three defects, two patients contributed two defects, and 18 patients contributed one infrabony defect each. The distribution of infrabony defects in relation to tooth type and treatment modality is presented in Table 1.

In both treatment groups, a slight decrease in FMPS, FMBS, PI, and GI was observed with regard to the baseline values. There was no statistically significant difference between treatment groups before treatment regarding the clinical and radiographic measurements ($p>0.05$). Table 2 presents the baseline and 18 months clinical and radiographic measurements of the two treatment groups. Statistically significant CAL gain, PPD reduction, and bone fill (CEJ-BD reduction) occurred in both groups 18 months after treatment ($p<0.05$). The mean crestal bone resorption (CEJ-AC) and the mean reduction of defect width showed no significant differences between baseline and 18 months for both treatment groups ($p>0.05$). Additionally, the change in defect angle and the defect resolution (AC-BD) between baseline and 18 months was not significant for the defects in the PRP-alone therapy group ($p>0.05$).

Table 1 Distribution of infrabony defects in relation to tooth type and treatment modality

		Anterior	Bicuspid	Molar	Total
DFDBA/PRP	Maxilla	8	1	0	16
	Mandible	0	3	4	
PRP	Maxilla	3	3	0	12
	Mandible	0	2	4	

Table 2 Mean and standard deviation of clinical and radiographic measurements of the two treatment groups (mean±SD)

Parameter	DFDBA + PRP		PRP	
	Baseline	18 months	Baseline	18 months
CAL-V	9.06±2	4.3±1.08*	8.4±1.3	6.9±1.3*
PPD	8.3±1.8	3.7±0.8*	7.5±1.3	5.4±1.3*
PI	0.31±0.6	0.25±0.4	0.33±0.8	0.25±0.5
GI	0.37±0.7	0.25±0.4	0.41±1	0.25±0.6
CEJ-AC	4.5±1.7	3.9±1.3	4.5±1.3	4.8±1.4
CEJ-BD	10.6±2.4	5.9±2.4*	9.5±1.3	8.7±1.5*
AC-BD	6±2.5	2.2±1.5*	4.7±1.09	4.06±1.2
Defect width	2.9±0.8	2.5±0.8	2.7±0.8	2.6±0.8
Defect angle (^{0})	25.9±10.9	36.7±10.7*	27.9±5.8	27.8±6.6

*$p<0.05$; difference is statistically significant within group.

The comparison of the two treatment groups regarding the mean gain in clinical and radiographic parameters is presented in Table 3. The DFDBA+PRP treatment presented more favorable gains in both clinical and radiographic parameters than the PRP-alone therapy group. The only exception occurred for the change in defect width and mean crestal bone resorption (CEJ-AC), which showed no statistically significant differences between the two treatment modalities ($p>0.05$).

Pearson correlation coefficient analysis showed important correlations within DFDBA+PRP combination; however, no statistically significant correlations were observed within the PRP treatment group. According to Pearson analysis, the baseline defect angle had an influence on CAL gain and bony fill. The amount of CAL gain ($p=0.001$, $r^2=-0.747$) and bony fill ($p=0.027$, $r^2=-0.550$) were much higher when the baseline defect angle was smaller. The initial defect depth was positively correlated to the bony fill ($p=0.047$, $r^2=0.503$), which indicated that the amount of bone fill increased with increasing defect depth.

When analyzed to compare the differences in clinical and radiographic changes between initially narrow and initially deep infrabony defects [≤18 ($n=4$)/≥33 ($n=4$) for DFDBA+PRP and ≤25 ($n=4$)/≥30 ($n=5$) for PRP], it was observed that there were no significant differences between narrow and wide defects in the PRP-alone therapy group

Table 3 Comparison of clinical and radiographic changes between the two treatment groups (mean±SD)

Parameter	DFDBA + PRP	PRP
CAL-V	4.6±1.5	1.5±0.7* (p=0.000)
PPD	4.6±1.2	2.1±0.5* (p=0.000)
CEJ-AC	0.6±1.5	−0.2±1.5 (p=0.47, 1−β=0.312)
CEJ-BD	4.6±2.2	0.8±0.8* (p=0.000)
AC-BD	3.8±2.3	0.6±1.2* (p=0.000)
Defect width	0.3±0.7	0.1±0.9 (p=0.32, 1−β=0.125)
Defect angle (^{0})	−10.8±10	0.08±6.1* (p=0.03)

*p<0.05; difference is statistically significant between the two treatment groups.

(Table 4). Nevertheless the differences between initially narrow and initially wide defects in the DFDBA + PRP group was significant regarding the mean CAL gain, PPD reduction, and defect resolution (AC-BD) in favor of the narrow defects (Table 4). As can be seen on Table 4, the difference between narrow and wide defects regarding the bone fill and the change in defect angle was much higher when the defect angle was "narrow." However, these differences failed to reach statistical significance (p>0.05).

Baseline and 18 months standardized radiographic images of infrabony defects treated with PRP alone and DFDBA + PRP combination are presented in Figs. 2a,b and 3a,b, respectively.

Discussion

This study compared DFDBA/PRP combination with PRP alone in the treatment of human infrabony defects. Our results indicate that DFDBA/PRP combination is an effective therapy modality for infrabony defects in patients with advanced chronic periodontitis. When compared with baseline values, statistically significant improvements in clinical and radiographic measurements occurred in both treatment groups. However, although significantly different from baseline values, the CAL gain and bone fill recorded on PRP-alone therapy group is fairly limited. The amount of bone fill observed in the PRP-alone treatment group is in accordance with other investigators who suggested that PRP is successful as a matrix enhancement factor with graft particles in combination therapies; however, a limited amount of healing is observed when it is used alone [1, 5, 26]. This could be due to the lack of an osteoinductive effect of PRP [35]. As a speculation, we may suggest that the amount of bone fill observed in the PRP-alone therapy group is consistent with routine flap surgery procedure, which usually results with 1 mm of regenerative gain at the most apical part of the bone defect [18]. The amount of defect fill and CAL gain observed after combination therapy in the present study is in accordance with previous studies where the benefits of the combined triple therapy (PRP/BPBM/GTR) in the treatment of human infrabony defects was shown [5, 26]. It was reported that the benefits of PRP/BPBM combination not only have statistical but also clinical significance [5]. A review of the literature shows that a mean threshold of approximately 60–65% bone fill can be achieved using DFDBA for the treatment of infrabony defects [17, 28, 34]. Quintero et al. [34] demonstrated an average bone fill of 65% after DFDBA therapy, which corresponded to a bone fill of 2.4 mm. The infrabony defect depth values, which were measured between the base of a stent that was used along with a calibrated periodontal probe and base of the defect, were lower than the present study. Therefore, it may be concluded that the differences between our study and Quintero et al. regarding the mean bone fill may be related to the differences between the preoperative CAL, PPD, and defect depth values and additionally the choice of material in two studies [23]. The DFDBA/PRP therapy resulted with an average bone fill of 3.8 mm in the present study, which is in accordance with previous reports where the clinical benefits of a regenerative approach employing a biologic

Table 4 Clinical and radiographic changes as related to baseline defect angle of infrabony defects in the two treatment groups (mean±SD)

Parameter	DFDBA + PRP		PRP	
	Defect angle≤18	Defect angle≥33	Defect angle≤25	Defect angle≥30
CAL-V	6.7±1.2	3.7±1.2* (p=0.03)	1.5±0.5	1.4±1.1 (p=0.89, 1−β=0.05)
PPD	6.2±0.9	3.7±0.9* (p=0.03)	2.5±0.5	2±0.7 (p=0.33, 1−β=0.16)
CEJ-AC	−0.1±0.8	1.8±1.7 (p=0.11, 1−β=0.40)	−0.05±0.5	−0.4±2.4 (p=0.90, 1−β=0.05)
CEJ-BD	7.05±1.7	3.7±1.4 (p=0.06, 1−β=0.68)	1.02±0.3	0.72±1.3 (p=0.70, 1−β=0.06)
AC-BD	7.02±2.2	1.9±0.3* (p=0.03)	0.5±1.02	0.7±1.7 (p=0.90, 1−β=0.05)
Defect width	−0.2±0.3	0.6±0.9 (p=0.14, 1−β=0.31)	−0.1±0.2	0.6±1.3 (p=0.27, 1−β=0.16)
Defect angle (^{0})	−21.2±13.4	−5.2±7.1 (p=0.11, 1−β=0.42)	−3.2±4.9	3.8±5.8 (p=0.10, 1−β=0.37)

*p<0.05; difference is statistically significant within the group.

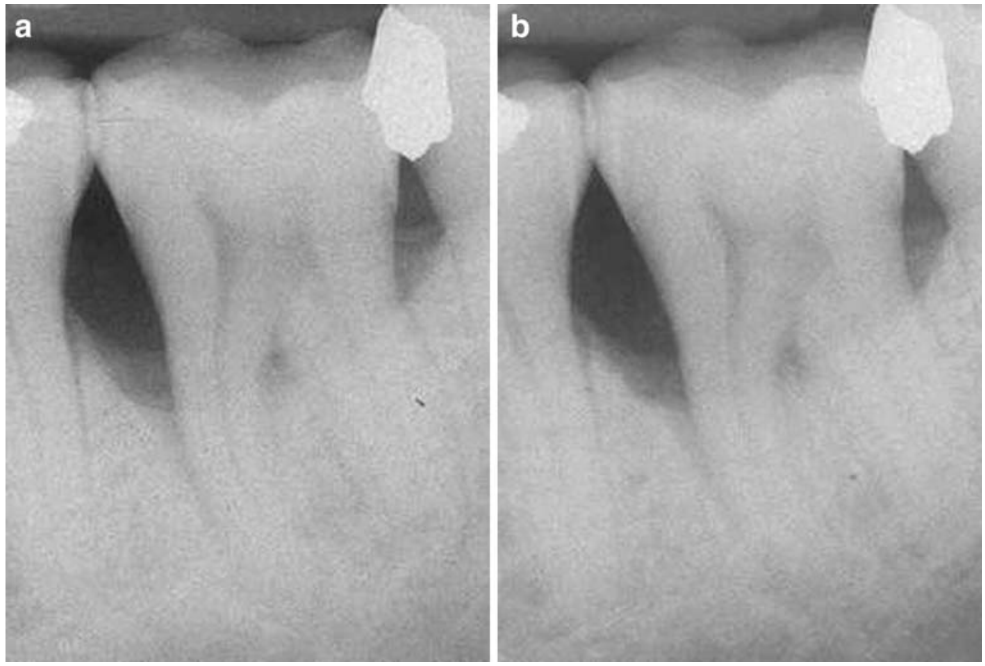

Fig. 2 **a** Baseline periapical radiographic image of the infrabony defect. **b** The infrabony defect 18 months after PRP-alone treatment

mediator combined with bone allografts was reported [4, 26]. It was shown that graft material has the ability to exert osteoconductive and/or osteoinductive effects [30, 31] and functioning as a matrix enhancement factor PRP can serve both in homeostasis and adhesion of graft material, as well as contribute physiologically to more rapid healing of the surgical site [6]. This assumption is based on the fact that two distinct wound-healing principles may be applied together in one clinical situation. However, the efficacy of the PRP-alone treatment compared to the DFDBA/PRP treatment was the primary aim of the present study. Our statements on the contribution of PRP to DFDBA/PRP combination have to be supported by a separate study in which DFDBA-alone therapy would be the object of investigation and will be compared to the DFDBA/PRP combination treatment.

It has been stated that infrabony defect characteristics may reflect the differences in clinical outcomes after periodontal therapies [23]. Several studies were performed to investigate the influence of baseline defect angle and

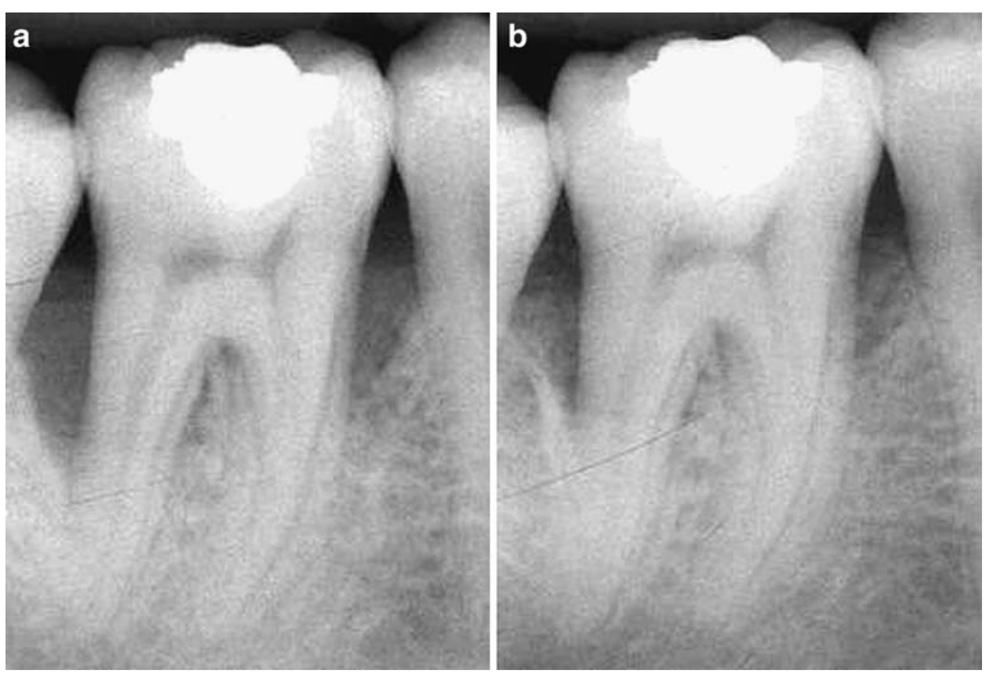

Fig. 3 **a** Baseline periapical radiographic image of the infrabony defect. **b** The infrabony defect 18 months after DFDBA/PRP combination treatment

defect depth by performing the same treatment procedure for infrabony defects with varying defect angles and depths. It was reported that the height and width of an infrabony defect may influence the results of periodontal surgery [9, 10, 22, 33]. However, the choice of material is also an important parameter, particularly for combination therapies, when predicting the treatment outcomes, and there is lack of information on how infrabony defects with similar characteristics respond to different treatment modalities. Since the infrabony defects included in the present study presented similar clinical and radiographic measurements, the only reason that could account for the differences in the treatment outcomes and the explanation for the lack of correlations or influence of baseline angle in one of our groups is the treatment modality. After DFDBA/PRP combination treatment, the correlation analyses showed that the CAL gain and bone fill were much higher when the infrabony defect was narrow and deep. The influence of defect depth and defect angle on the outcomes was reported before after GTR [22] and EMD [42] treatment. However these correlations were not observed in PRP alone therapy group which presented limited improvement in both clinical and radiographic parameters. Our results regarding bone fill, CAL gain and the increase in defect angle in DFDBA/PRP treatment group are complementary and can be defined as improved bone condition in infrabony defects.

Previous studies tried to determine cutoff values to help clinicians when selecting the ideal cases for regeneration. Cortellini and Tonetti defined the radiographic defect angles ≤25 as narrow and angles ≥37 as wide using the 25th and 75th percentages for the infrabony defects treated with guided tissue regeneration [10]. Using enamel matrix derivative, Tsitoura et al. [42] suggested that these “universal” cutoffs may be used in clinical practice. The present authors decided that the interquartile range should be performed separately for the two groups to evaluate whether the choice of material influences the differences between narrow/wide defects. However, the sample size became a deficiency when the infrabony defects were classified as narrow or wide according to interquartile ranges. This led to a dramatic decrease in the number of defects to be compared as narrow and wide for both of the groups. The cutoff values were different for the two treatment groups, which were also both different than the values reported by other investigators. The cutoff values for narrow and wide infrabony defects were determined as ≤18/≥33 for DFDBA/PRP combination and ≤25/≥30 for the PRP-alone treatment groups in the present study. The difference between the cutoff values from previous studies and within the two treatment modalities may be related to the limited number of infrabony defects included in the present study and suggests the need for performing an interquartile range to determine narrow and wide defects when small clinical trials are the case. Our results showed a significant difference between narrow and wide defects regarding CAL gain, PPD reduction, and defect resolution in favor of the narrow defects in the DFDBA/PRP combination group. This may represent the difference between the healing potential of narrow and wide defects. The wider defects may present a healing challenge since more tissue is lost and the superficial component of a wide defect may be exposed to the adverse effects of the oral environment [39]. According to our results, the differences between narrow and wide defects regarding the clinical and radiographic measurements were nonsignificant for the PRP-alone treatment group, which indicates the need for a larger sample size according to the power calculation. It should be noted that the cutoff value for narrow defects in the PRP-alone group is higher than it is for the defects in the DFDBA + PRP group and this may also account for the nonsignificant differences between narrow and wide defects in the PRP group. Therefore, a further study with a larger sample size is definitely needed to investigate the influence of PRP on the treatment outcomes of narrow/wide defects. Nevertheless, a significant influence of baseline angle on clinical and radiographic outcomes was shown in the DFDBA/PRP group, which had a similar sample size as the PRP-alone group when classified according to interquartile ranges. The limited amount of healing, lack of correlations, and nonsignificant differences between narrow and wide defects in the PRP-alone group may also suggest the importance of the choice of material that is in accordance with the defect characteristics when predicting clinical and radiographic outcomes.

Gunsolley et al. [19] has reported that a sample size ranging from 64 to 137 per treatment, much larger than ours, is necessary to definitively show a statistically significant difference between the two treatment modalities. These are definitely unrealistic numbers for single-centered clinical investigations and were the primary reason why the study design was connected to a deadline rather then performing a power analyses. The number of infrabony defects included in the present study was inconsistent with many clinical studies [16, 36, 38]. The selection of patients to the two treatment groups was performed according to the date that he/she had applied to the clinic. Although this method is not the ideal one, this was the most appropriate randomization method that was compatible with our clinical schedule and study protocol, and we are in the opinion that this deficiency would not change the overall trend of this study. Some patients’ contribution of more than one infrabony defect may rise as suspect as defect characteristics, patient characteristics, and the treatment protocol are the primary factors that influences the treatment outcomes of biomaterial protocols [7, 8, 39, 40]. However, there were only four patients with more than one infrabony defect, and considering our limited study sample, we performed nonparametric statistical tests to analyze the data. Further multicentered studies with larger sample sizes and considering individual differences are needed to confirm these results.

Because of the concentration on the defect and biomaterial characteristics and the negligence of the patient factor, as some of them contributed more than one defect due to the study design, the examiner being aware of the treatment modality before surgery can be considered as deficiencies of the present study. With single-centered pilot studies involving limited numbers, it is a challenge for the clinicians to overcome the problems associated with the study design. Nevertheless, the number of infrabony defects included was sufficient enough to present the superiority of the DFDDBA/PRP combination on the PRP-alone treatment regarding the clinical and radiographic improvements. A significant influence of the baseline defect angle on the treatment outcomes for the infrabony defects treated with the DFDBA/PRP combination was also shown. Further studies are needed to evaluate the influence of defect characteristics on the outcomes of the DFDBA/PRP combination and, especially, the PRP-alone therapy, while determining the contribution of PRP to the DFDBA/PRP treatment with an additional DFDBA-alone therapy group.

In conclusion, the results of this study indicate that the DFDBA/PRP combination is more effective than PRP alone in the treatment of human infrabony defects, and the amount of CAL gain, PPD reduction, and bone fill increases when the infrabony defect is narrow and deep before the DFDBA/PRP combination treatment.

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