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ORIGINAL ARTICLE

A clinical comparison of autogenous bone graft with and without autogenous periodontal ligament graft in the treatment of periodontal intrabony defects

Adileh Shirmohammadi · Mohammad Taghi Chitsazi · Ardeshir Lafzi

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Abstract The aim of the present study was to evaluate the efficacy of autogenous bone graft (ABG) with and without autogenous periodontal ligament graft (PDLG) in the management of human two-wall intrabony periodontal defects. Twenty-six similar two-wall intrabony periodontal defects with ≥5 mm probing depths and ≥3 mm depths of intrabony component in 13 nonsmoking healthy patients were selected. One defect in each subject was treated with ABG alone (ABG group) and the contralateral one with ABG and PDLG (PDLG group). The primary outcomes of the study included changes in clinical probing depth (CPD) and clinical attachment level (CAL). Groups showed statistically significant improvements in soft and hard tissue parameters after 6 months. However, the between-group differences after 6 months were not statistically significant with regard to soft and hard tissue measurements except CAL gain. In the combined group, it was significantly higher than the ABG group (3.69 and 2 mm, respectively; P=0.03). Within the limits of this study, both treatments resulted in marked clinical improvement, but combined treatment seemed to enhance the results in the treatment of two-wall intrabony defects.

Keywords Intrabony defect · Bone graft · Periodontal regeneration · Autogenous bone graft · Periodontal ligament

A. Shirmohammadi · M. T. Chitsazi (✉) · A. Lafzi
Periodontics Department, Tabriz Dental Faculty,
Golgasht St.,
Tabriz, Iran
e-mail: chitsazm@tbzmed.ac.ir

Introduction

The aim of periodontal therapy is to treat the infection caused by bacterial plaque and to arrest further disease progression and tissue damage. Periodontal regeneration is defined as the reconstruction of the damaged periodontium as evidenced histologically by the formation of new cementum, periodontal ligament, and alveolar bone to a previously diseased root surface [33], which may only be accomplished in certain well-defined cases. Several clinical procedures have been used to this end so far, including bone grafts [28, 34], guided tissue regeneration (GTR) [6, 38], application of growth factors [30], application of enamel matrix derivatives [10], or combinations of these procedures [14, 35].

It is purported that new bone formation (up to 30% bone fill) regularly occurs in surgically treated intrabony defects. Studies indicate that additional use of bone grafts results in greater levels of bony infill to a maximum of 60–70%. The extent of gain in new attachment is very variable with bone grafts, but autogenous bone graft (ABG) has been used with success for some years [24].

Various studies have demonstrated that new attachment and regeneration of periodontium may be facilitated when the healing area is selectively populated with PDL cells [15, 16]. It is evident that cultured PDL cells have the potential to differentiate into osteoblasts or cementoblasts and promote formation of PDL, alveolar bone, and cementum in vitro [3, 21]. In addition, animal studies have shown that cultured PDL cells are capable of synthesizing periodontal tissue after replantation in vivo [4, 8, 20]. In a study recently carried out by Akbay et al., autogenous periodontal ligament graft (PDLG) was used in the treatment of furcation lesions [2]. This study suggests that the use of PDL grafts has beneficial effects in the treatment of furcation defects. Since the

benefits brought by adding PDLG to ABG are not clear yet, the purpose of the present study was to compare the clinical outcomes obtained by the combination of ABG and PDLG with those obtained by ABG alone as a therapy for human intrabony defects.

Materials and methods

Study population and experimental design

In this split-mouth study, 13 patients (seven females and six males with a mean age of 33±8 years), who had referred to the Periodontics Department of Tabriz Dental Faculty for the treatment of periodontal disease, were included.

Medical and dental histories were reviewed for the following exclusion criteria: (1) Any systemic condition that would preclude periodontal surgery; (2) pregnant women or nursing mothers; (3) full-mouth plaque score [29] and full-mouth bleeding score [1] above 20%; (4) patients who had received antibiotic therapy in the past 6 months; (5) patients who were under orthodontic therapy (a history of orthodontic therapy was not considered a preventive factor for inclusion); (6) smoking.

Once the patients had been screened for any exclusion criteria, they were considered eligible to take part in the study based on the following criteria: (1) At least one pair of matched two-wall intrabony periodontal defect with a probing depth of ≥5 mm and ≥ 3 mm depth of intrabony component (depth of intrabony component and number of bony walls) following phase I therapy and re-evaluation; (2) the existence of one fully erupted third molar with healthy periodontium.

The study design was approved by the Ethics Committee and supported by the Research Deputy of Tabriz Medical Sciences University. The nature of this investigation was explained in detail, and the patients signed an informed consent form. All the patients underwent initial therapy, consisting of oral hygiene instructions, full-mouth scaling and root planing, correction of restorations, restoration of decayed teeth, and occlusal adjustment when indicated.

Plaque control was assessed at each scaling, and root planing appointment. Four weeks after the completion of initial therapy, re-evaluation examination was performed.

At the time of surgery full-mouth plaque score, full-mouth bleeding score and gingival index [23] were recorded, and clinical parameters were measured with a Williams periodontal probe (PWD, Hu-Friedy Immunity, USA) from a fixed point using customized acrylic stents to prevent angulation and positioning errors. All baseline clinical parameters were obtained on the day of the surgery by one examiner, who was blind to the type of treatment. Final parameters were taken 6 months postoperatively by the same examiner, again blind to the method of the study. A calibration exercise was performed to obtain acceptable intra-examiner reproducibility for probing depth and recession of the gingival margin. Prior to the study and after 6 months, five patients, each with ten teeth with probing depth of >5 mm on at least one aspect of each tooth, were used for calibration. The examiner evaluated the patients on two occasions, 48 h apart. Calibration was accepted if >90% of the recording could be reproduced within a 1.0-mm difference.

The following soft tissue measurements were included:

1. Clinical probing depth (CPD): free gingival margin to the base of the pocket
2. Clinical attachment level (CAL): inferior margin of the stent to the base of the pocket
3. Gingival margin level (ST-GM): inferior margin of the stent to the free gingival margin. Differences between the baseline measurements and those obtained 6 months after surgery demonstrated gingival recession.

Surgical procedure

A single surgeon (A S) performed all the operations. Surgical sites were anesthetized utilizing 0.2% lidocaine with 1:80,000 epinephrine. Following buccal and lingual sulcular incisions, full-thickness flaps were raised. Granulation tissue was removed to allow visualization of the defect. Root surfaces were scaled and root planed by hand and ultrasonic instrumentation.

Hard tissue measurements were made with the same stent:

1. Inferior margin of the stent to the alveolar crest (Stent-AC)
2. Inferior margin of the stent to the base of the defect (Stent-DB)
3. Alveolar crest to the bottom of the defect (INFRA)

Comparisons were carried out between the baseline measurements and those obtained 6 months later to determine alveolar crest resorption, defect fill and defect resolution, respectively.

One defect from each pair of intrabony defects was randomly selected, by the flip of a coin, to be treated with autogenous bone graft with or without autogenous periodontal ligament graft (ABG or ABG/PDLG). Cortical bone chips were taken from the surgical sites or edentulous sites by hand instruments.

Fully erupted third molars were extracted with forceps using only gentle rotating movements when possible and kept in a sterile saline solution with tetracycline (50 mg/ml). PDL remnants attached to the cementum and cellular cementum were removed using curettes. To circumvent contamination by gingival fibroblasts, the most coronal 4 mm of tissue on the

root surface was removed by sharp dissection and root planning. PDL cells from the middle third of the roots were used [2]. The walls of the defect were covered with a PDL graft, and then, the inner part of the defect was filled with ABG.

The defects were overfilled with bone grafts and then tightly packed using amalgam condensers to the level of the surrounding bony walls. Closure was accomplished using 4-0 sutures in vertical mattress fashion and a periodontal dressing (Coe-Pak, GC America, IL, USA) was used.

Postoperative care

The patients were instructed to rinse twice daily for 4 weeks with a 0.2% solution of chlorhexidine gluconate. Acetaminophen was prescribed for postoperative pain and Amoxicillin 500 mg tid was administered for 10 days.

The patients were re-visited after 7 days for removal of the periodontal dressing and sutures. At the end of 2 weeks and then every month, the patients received professional prophylaxis and oral hygiene reinforcement.

Six months after the primary surgery, a reentry surgery was performed. The reentry procedure was aimed at correction of any remaining defects and evaluation of the results of the treatment with reference to the soft and hard tissue parameters. The method of reentry measurements was similar to primary measurements.

Statistical analysis

The primary outcomes of the study were CPD reduction and CAL gain, whereas gingival recession, defects fill, crestal resorption, and defect resolution were the secondary outcomes. The control variables were FMPS, FMBS, and GI.

Sample size was determined by comparing the two means. By considering α=0.05, d=0.1 with 80% power and CPD mean value of 7.01±1.67 for PDLG group and 7.58±1.17 for ABG group (which were yielded by a pilot study), the sample size was determined to be 11 patients (22 defects), but we used 13 patients (26 defects) to increase the validity of the results.

The patient was defined as the statistical unit. Statistical analysis was performed using the descriptive statistic methods (mean±SD), repeated measurements analysis of variance (nonparametric) and Wilcoxon test, using SPSS version 14 software. The normality of data distribution was evaluated using Kolmogorov–Smirnov test.

Results

A total of 13 patients with 13 pairs of intrabony defects received surgical therapy. A total of 13 sites were treated with ABG (ABG group) Fig. 1, and 13 sites were treated with ABG/PDLG (PDLG group, Fig. 2). Postoperative healing was uneventful in all cases, and no complications or infections were observed throughout the study period.

All subjects returned for re-evaluation after 6 months. During the 6-month period following treatment, all the patients maintained excellent levels of oral hygiene, and full-mouth plaque score, full-mouth bleeding score and gingival index did not demonstrate any significant differences compared to the baseline values (P>0.05; Table 1). There were no significant differences in the initial measurements of soft and hard tissue parameters between ABG and PDLG groups (Tables 2 and 3).

Soft tissue parameters

Soft tissue parameters are presented in Table 2. In comparison with the baseline data, both the test and control groups showed statistically significant differences. Clinical probing depth reduction in the ABG group was 2.84±0.89 mm (P<0.001) and 4.07±0.95 mm in the PDLG group (P<0.001). However, the between-group differences after 6 months were not statistically significant (P=0.16). The clinical attachment gains were 2±0.91 mm in the ABG group and 3.69±0.75 mm in the PDLG group. Both the within-group differences were significant (P<0.001). The difference between the groups was in favor of the PDLG group (P=0.03).

An average change of 0.69±0.75 mm in the position of the gingival margin between the baseline and 6-month data was observed in the test group (P<0.05) and 0.76±0.43 mm in the control group (P<0.05). In both groups, recession showed a tendency to increase, but the postoperative recession was not affected by the treatment protocol (P=0.85).

Hard tissue parameters

Hard tissue parameters are presented in Table 3. In comparison with the baseline data, both the test and control groups showed statistically significant differences. An evaluation of the hard tissue findings indicated that both treatment modalities result in defect fill after 6 months. The PDLG group showed 2.92 mm (P=0.005) of defect fill, while this was 2.38 in the ABG group (P=0.014); however, the between-group differences after 6 months were not statistically significant (P>0.05).

Alveolar crest resorption increased significantly: 0.53±0.51 mm in the ABG group (P=0.002) and 0.69±0.48 mm in the PDLG group (P=0.009), but there were no significant differences between the two groups after 6 months.

Defect resolution was better in the PDLG group (3.61±1.44 mm) compared to that in the ABG group (2.92±

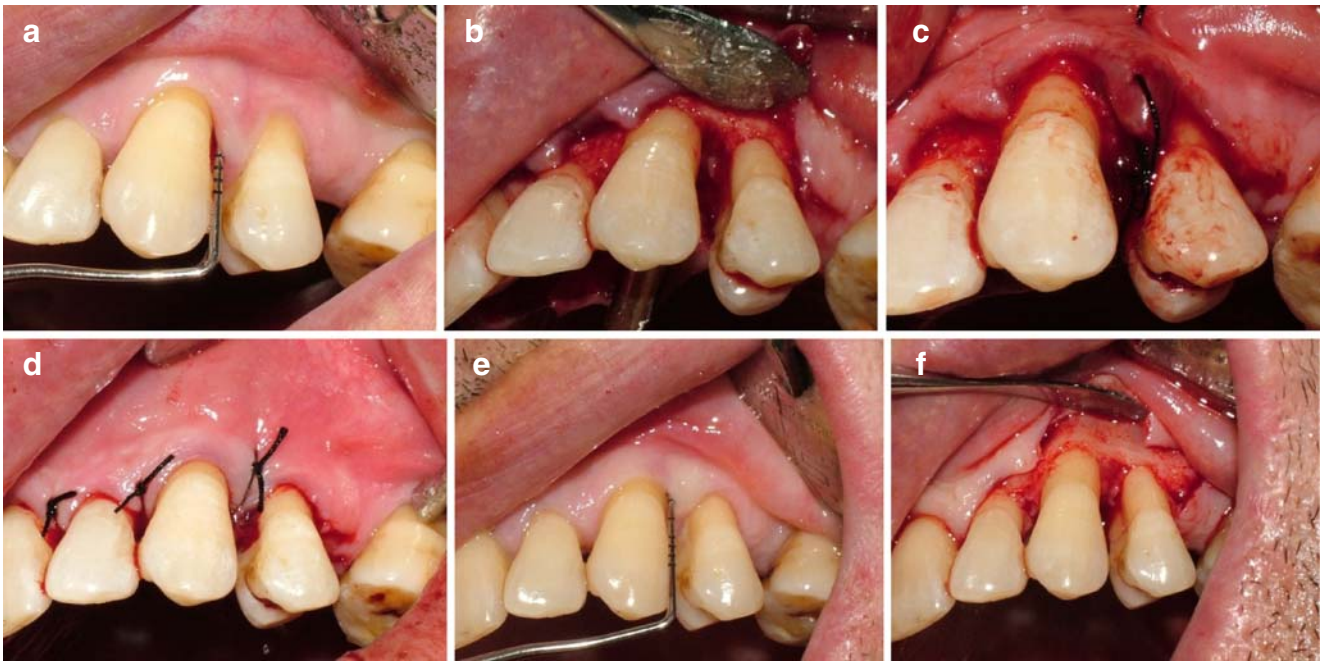

Fig. 1 Case from ABG group: **a** Clinical appearance of the intrabony defect at tooth 23 at the time of surgery. **b** Appearance of the defect. **c** Placement of the autogenous bone graft. **d** Suturing. **e** Clinical appearance at 6 months posttreatment. **f** Reentry

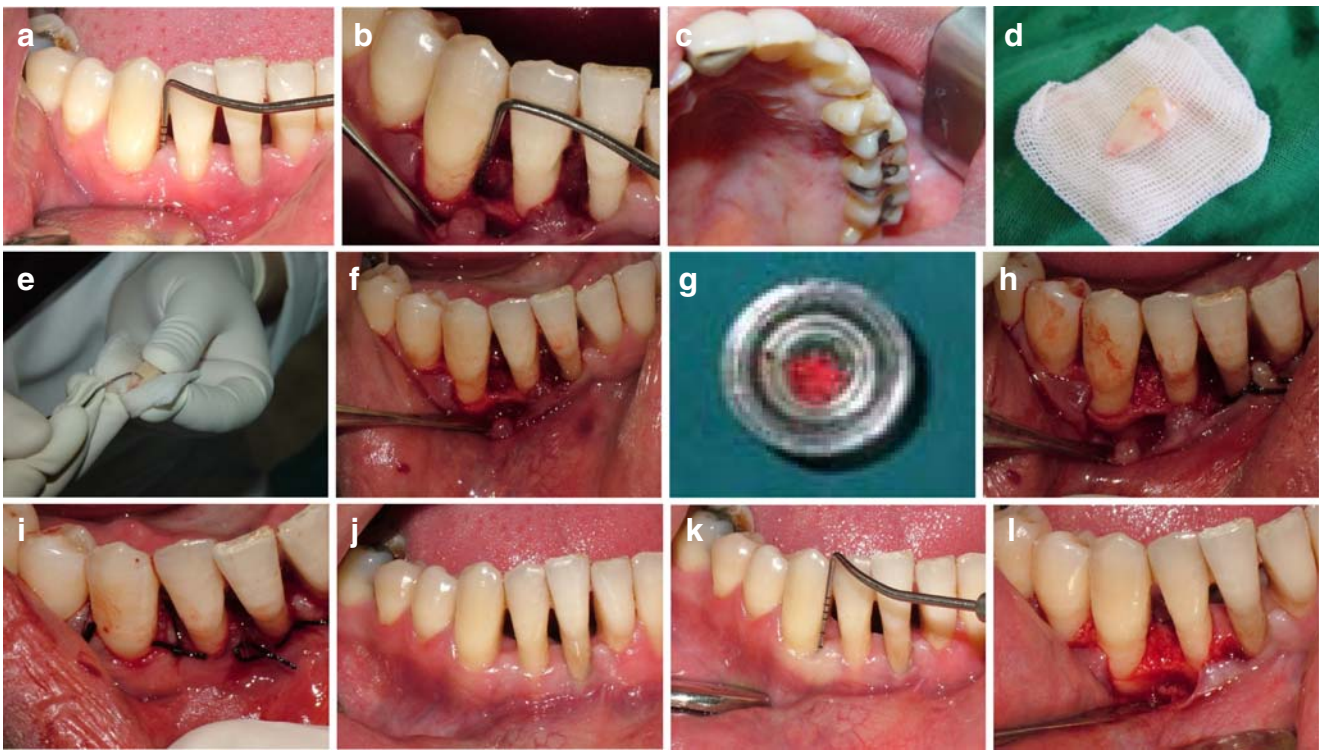

Fig. 2 Case from PDLG group: **a** Clinical appearance of the intrabony defect at tooth 43 at the time of surgery. **b** Appearance of the defect. **c** Fully erupted third molar. **d** Extracted third molar. **e** preparation of periodontal ligament autograft. **f** Placement of it. **g** The autogenous bone graft. **h** Placement of it. **i** Suturing. **j**, **k** Clinical appearance at 6 months posttreatment **l** Reentry

Table 1 Mean (± SD) of FMPS, FMBS and GI at baseline and after 6 months

	PDLG Group	ABG Group	*p* value
FMPS			
Baseline	8.2%±2.7%	9.2%±1.6%	0.500
6 months	7.5%±2.4%	8.8%±3.7%	0.345
p value	0.673	0.844	
FMBS			
Baseline	7.2%±2%	8.2%±3.6%	0.673
6 months	8.1%±2.9%	9.8%±1.7%	0.802
p value	0.400	0.512	
GI			
Baseline	0.8±0.6	0.8±0.6	1.000
6 months	0.9±0.8	1.0±0.3	0.768
p value	0.982	0.765	

0.95 mm). The between-group differences were not statistically significant (P=0.08).

Discussion

Regeneration of lost attachment apparatus is the treatment of choice for intrabony defects in contemporary clinical practice. The present experimental study was undertaken to evaluate the clinical effectiveness of ABG alone or combined with PDL autograft in the treatment of intrabony periodontal defects. The two treatment modalities in the present study resulted in clinically significant improvements in all the soft and hard tissue parameters when baseline data and 6-month data were compared.

Furthermore, statistical analysis of data revealed no significant differences between the two treatment modalities with regard to soft and hard tissue measurements except for clinical attachment level. The mean CAL gain as observed 6 months postoperatively was 3.69±0.75 mm in the PDLG group and 2±0.91 mm in the ABG group. In this context, it should be pointed out that these are the first data obtained for the evaluation of the use of PDL autograft for the treatment of intrabony periodontal defects. Therefore, a comparison with other studies is not possible. However, the CAL gains noted in the PDLG group seemed to be within the range of other well-documented regenerative treatment procedures, but the CAL gains in the ABG group is moderate and comparable with most of the reported results from open flap debridement [22, 31]. Nevertheless, one systematic review comparing the autogenous bone to the OFD procedure indicated a greater clinical attachment level gain for the grafted group (clinical attachment level gain 3.2 mm, SD 0.5) [36].

The differences in PD at baseline between the groups in our study (2.85±0.89 mm in the ABG group and 4.07±0.95 mm in the PDLG group) are consistent with the results of related studies.

Guillemin et al. reported a 2.3-mm PPD reduction and a 3.2-mm gain in CAL [12]. According to the results of a meta-analysis evaluating grafting materials, the use of biological agents in periodontal intrabony defects produces a favorable change in PPD and CAL values when compared with an access flap procedure [36]. Nevertheless, there appeared to be a marked variation in CAL gain and PPD reduction with respect to different biomaterials or even between studies valuating the same biological agent. In a study which has recently been performed by Akbay et al., autogenous periodontal ligament graft (PDLG) was used in the treatment of furcation lesions [2]. Sites treated with PDL grafts demonstrated significant improvements in vertical and horizontal defect fill, PD, and CAL at 3 and 6 months compared to presurgical values.

It has been documented that there are several prognostic factors that affect the outcome of regenerative procedures, including type of the defect treated (initial PDs and attachment level, width, depth and angle of defects, intrabony wall components), type of barrier membrane (different cross-linking techniques) or grafts used (biological and physicochemical characteristics of bone grafts), operator's experience, surgical variables and methods, measuring techniques, postoperative maintenance, and statistical analysis. In addition to these, other factors associated with bacterial contamination, innate wound healing potential, and the surgical procedure affect the treatment outcome [18].

Table 2 Soft tissue parameters at baseline and after 6 months

Variable	Group	Baseline value (mm)	Baseline comparison	6-month value (mm)	Within-group comparison	Between-group comparison
CPD	PDLG	7.23±1.09	P=0.43	3.08±0.27	P<0.001*	P=0.16
	ABG	6.31±1.49		3.46±0.66	P<0.001*	
CAL	PDLG	10.15±0.98	P=0.61	6.54±0.77	P<0.001*	P=0.03*
	ABG	9.54±1.33		7.54±1.05	P<0.001*	
Stenet-GM	PDLG	0.55±2.85	P=0.27	3.62±0.96	P<0.001*	P=0.85
	ABG	1.01±3.23		4±1.08	P<0.001*	

Asterisk indicates statistical significance based on P<0.05.

Table 3 Hard tissue parameters at baseline and after 6 months

Parameter	Group	Baseline value (mm)	Baseline comparison	6-month value (mm)	Within-group comparison	Between-group comparison
Stenet-AC	PDLG	8.92±1.55	P=0.81	9.62±1.75	P=0.009*	P=0.34
	ABG	8.85±1.51		9.38±1.85	P=0.002*	
Stenet-DB	PDLG	13.15±1.95	P=0.91	10.15±1.86	P=0.005*	P=0.69
	ABG	12.92±1.97		10.54±1.94	P=0.014*	
INFRA	PDLG	4.15±1.21	P=0.35	0.54±0.51	P=0.003*	P=0.08
	ABG	4.08±1.18		1.15±0.68	P=0.004*	

Asterisk indicates statistical significance based on $P<0.05$.

The intrabony component of the defect in the present study decreased by an average of 2.92 and 3.61 mm in the ABG and PDLG groups, respectively. The within-group differences 6 months postoperatively were significant. Because the amount of crestal bone resorption was minimal, these changes mostly reflect the filling of the intrabony defect.

The results of this study indicated that combined treatment was superior in promoting defect fill when compared to presurgical levels. A surgical reentry of the treated defects revealed a greater amount of defect fill in favor of the PDLG group (2.92 mm) compared to the ABG group (2.38 mm). In clinical case series, in which intraoral autogenous grafts were used for the treatment of intrabony periodontal defects, a mean bone fill ranging from 3 to 3.5 mm was reported [11, 13, 27, 32].

The mean bone fill in Froum's [11] study using bone blending was 2.98 mm and in Hiatt and Schalhorn [13] study using intraoral cancellous bone was reported 3.5 mm. In this study, the mean bone fill after using ABG was 2.38 mm. The differences between this study and other studies might be attributed to the type of autogenous bone, the type of osseous defects, and the greater initial defect depth.

The treatment of intrabony defects with various grafting materials has provided a baseline for what can be achieved with regard to regenerative efforts to create bone fill. New attachment and regeneration of periodontium may be facilitated when the healing area is selectively repopulated with PDL cells. It is evident that cultured PDL cells have the potential to differentiate into osteoblasts and promote formation of PDL, alveolar bone, and cementum in vitro [5, 26]. In addition, animal studies have shown that cultured PDL cells are basically capable of synthesizing periodontal tissue after replantation in vivo [4, 8, 20].

Van Dijk et al. [37] created artificial periodontal defects on the buccal aspects of the lower second, third, and fourth premolars in a beagle dog. The exposed roots were thoroughly planed, and the cultured cells at passage 5, removed from an upper premolar, were applied on the planed root surfaces. The same treatment was administered on the planed surfaces of control teeth except for the fact that they were not seeded with PDL cells. Four months after implantation, histological sections were obtained. New connective tissue attachment was observed at test site. Epithelial cells from the gingival tissue did not grow downward into the planed root surface, and root resorption was not observed.

Lang et al. [21] obtained primary cell cultures from alveolar bone and PDL of minipigs and replanted these cells into experimentally induced furcation and interdental defects along with a carrier material made of bone gelatin and covered with Teflon membranes. When the defects were histologically assessed after 90 days, it was demonstrated that replantation of cultured alveolar bone cells had led to the formation of much more attachment and bone than control groups [flap surgery, bone gelatin (carrier material) and membrane group; flap surgery and membrane group; flap surgery group; and no treatment group]. Periodontal regeneration is based on the selective proliferation of cells originating from the periodontal ligament and bone while preventing the proliferation and migration of basal epithelial cells of gingival epithelium. Histological analysis of human periodontal defects treated with some bone grafts has revealed healing by a long junctional epithelium with minimal new connective tissue attachment and minimal new bone formation [7, 9]. In our experimental study, the walls of the defect were covered with PDL graft so that epithelial cells from the gingival tissue presumably do not grow downward into the planed root surface, although confirmation of the type of healing requires histological study.

Another factor that might influence treatment outcomes is storage of extracted third molars in a sterile saline solution with tetracycline. Placing PDL samples along with tetracycline in the bony defect might have confounded the results in the test group. Alger et al. [3] showed that there was a trend for greater connective tissue attachment following tetracycline–HCl treatment of roots. However, another study suggests that there is no significant benefit from reconstituting grafts with tetracycline [25].

In our study, recession was seen in both groups after 6 months (0.69±0.75 mm in the PDLG group and 0.76±0.43 in the ABG group), which is in accordance with other regenerative procedures [17, 19]. Nevertheless, the results of this study failed to demonstrate the superiority of one form of the treatment over the other (P=0.85).

One of the limitations in this study was the sample size. A larger sample size might have demonstrated statistically significant differences between the two groups. Furthermore, the large variability in patient response to therapy created large SDs which limited the ability for statistical analysis to demonstrate differences between the study groups.

Future research might be directed toward the ultrastructural assessment of mechanisms underlying the clinical events. In addition, investigation into the simultaneous healing of periodontal soft and hard tissues affected by the periodontal autograft and the mutual interactions of these two with reference to mediator molecules and other regional factors seem interesting.

Within the limits of the present study, it can be concluded that: (1) 6 months after surgery both therapies resulted in statistically significant PD reductions, CAL gains, and bone fill; (2) treatment with PDL autograft resulted in relatively higher CAL gain compared to treatment with autogenous bone graft alone.

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References

1. Ainamo J, Bay I (1975) Problems and proposals for recording gingivitis and plaque. Int Dent J 25:229–235
2. Akbay A, Baran C, Günhan O, Ozmeriç N, Baloş K (2005) Periodontal regenerative potential of autogenous periodontal ligament grafts in Class II furcation defects. J Periodontol 76:595–604
3. Alger FA, Solt CW, Vuddhakanok S, Miles K (1990) Histologic evaluation of new attachment in periodontally diseased human roots treated with tetracycline-hydrochloride and fibronectin. J Periodontol 61:447–55
4. Andreasen JO, Kristerson L (1981) Evaluation of different types autotransplanted connective tissues as potential periodontal ligament substitutes. An experimental replantation study in monkeys. Int J Oral Surg 10:189–201
5. Arceo N, Sauk JJ, Moehring J, Foster RA, Somerman MJ (1991) Human periodontal cells initiate mineral-like nodules in vitro. J Periodontol 62:499–503
6. Becker W, Becker BE, Mellonig J, Caffesse RG, Warrer K, Caton JG, Reid T (1996) A prospective multi-center study evaluating periodontal regeneration for class II furcation invasions and intrabony defects after treatment with a bioresorbable barrier membrane: 1-year results. J Periodontol 67:641–649
7. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E (1989) Histologic evaluation of new attachment apparatus formation in humans. Part I.J Periodontol 60(12):664–74
8. Boyko GA, Melcher AH, Brunette DM (1981) Formation of new periodontal ligament by periodontal ligament cells implanted in vivo after culture in vitro. A preliminary study of transplanted roots in the dog. J Periodontal Res 16:73–88
9. Cetinkava BO, Keles GC, Ayas B, Aydin O, Kirtiloglu T, Acikgoz G (2007) Comparison of the proliferative activity in gingival epithelium after surgical treatments of intrabony defects with bioactive glass and bioabsorbable membrane. Clin Oral Investig 11:61–68
10. Chitsazi MT, Mostofi Zadeh Farahani R, Pourabbas M, Bahaeddin N (2007) Efficacy of open flap debridement with and without enamel matrix derivatives in the treatment of mandibular degree II furcation involvement. Clin Oral Investig 11:385–389
11. Froum SJ, Gomez C, Breault MR (2002) Current concepts of periodontal regeneration. A review of the literature. N Y State Dent J 68:14–22
12. Guillemin MR, Mellonig JT, Brunsvold MA (1993) Healing in periodontal defects treated by decalcified freeze-dried bone allografts in combination with e-PTFE membranes. (I). Clinical and scanning electron microscope analysis. J Clin Periodontol 20:528–536
13. Hiatt WH, Schallhorn RG (1973) Intraoral transplants of cancellous bone and marrow in periodontal lesions. J Periodontol 44:194–208
14. Ilgenli T, Dündar N, Kal BI (2007) Demineralized freeze–dried bone allograft and platelet-rich plasma vs. platelet-rich plasma alone in infrabony defects: a clinical and radiographic evaluation. Clin Oral Invest 11:51–59
15. Isidor F, Karring T, Nyman S, Lindhe J (1986) The significance of coronal growth of periodontal ligament tissue for new attachment formation. J Clin Periodontol 13:145–150
16. Karring T, Isidor F, Nyman S, Lindhe J (1985) New attachment formation on teeth with a reduced but healthy periodontal ligament. J Clin Periodontol 12:51–60
17. Kasaj A, Röhrig B, Reicher C, Willershausen B (2008) Clinical evaluation of anorganic bovine-derived hydroxyapatite matrix/cell-binding peptide (P-15 in the treatment of human infrabony defects. Clin Oral Invest 12:241–247
18. Kornman KS, Robertson PB (2000) Fundamental principles affecting the outcomes of therapy for osseous lesions. Periodontoloy 22:22–43
19. Kuru B, Yılmaz S, Argın K, Noyan U (2006) Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects. Clin Oral Invest 10:227–234
20. Lang H, Schüler N, Arnhold S, Nolden R, Mertens T (1995) Formation of differentiated tissues in vivo by periodontal cell populations cultured in vitro. J Dent Res 74:1219–25
21. Lang H, Schüler N, Nolden R (1998) Attachment formation following replantation of cultured cells into periodontal defects-a study in minipigs. J Dent Res 77:393–405
22. Lang NK (2000) Focus on intrabony defects-conservative therapy. Periodontol 22:51–58
23. Löe H, Silness J (1963) P. J Periodontol 38:610–616 (Suppl) periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 22:533–551
24. Manson JD, Ely BM (2002) Outline of periodontics, 4th ed. Elsevier, Oxford, p 292
25. Masters LB, Mellonig JT, Brunsvold MA, Nummikoski PV (1996) A clinical evaluation of demineralized freeze-dried bone allograft in combination with tetracycline in the treatment of periodontal osseous defects. J Periodontol 67:770–81

26. Mukai M, Yoshimine Y, Akamine A, Maeda K (1993) Bone-like nodules formed in vitro by rat periodontal ligament cells. Cell Tissue Res 271:453–460
27. Nabers CL, O'Leary TJ (1965) Autogenous bone transplants in the treatment of osseous defects. J Periodontol 36:5–14
28. Nevins ML, Camelo M, Rebaudi A, Lynch SE, Nevins M (2005) Three-dimensional micro-computed tomographic evaluation of periodontal regeneration: a human report of intrabony defects treated with Bio-Oss collagen. Int J Periodontics Restorative Dent 25:365–373
29. O'Leary TJ, Drake RB, Naylor JE (1972) The plaque control record. J Periodontol 43:38–42
30. Ouyang XY, Qiao J (2006) Effect of platelet-rich plasma in the treatment of periodontal intrabony defects in humans. Chin Med J (Engl) 119:1511–1521
31. Pontoriero R, Wennström J, Lindhe J (1999) The use of barrier membranes and enamel matrix proteins in the treatment of angular bone defects. A prospective controlled clinical study. J Clin Periodontol 26:833–840
32. Robinson RE (1969) Osseous coagulum for bone induction. J Periodontol 40:503–10
33. Sculean A, Stavropoulos A, Berakdar M, Windisch P, Karring T, Brecx M (2005) Formation of human cementum following different modalities of regenerative therapy. Clin Oral Investig 9:58–64
34. Sperling I, Kozlovsky A, Tal H (1994) Contiguous autogenous transplant-nineteen years' clinical and radiographic follow-up: a case report. Quintessence Int 25:555–560
35. Stavropoulos A, Karring T (2005) Five-year results of guided tissue regeneration in combination with deproteinized bovine bone (Bio-Oss) in the treatment of intrabony periodontal defects: a case series report. Clin Oral Invest 9:271–277
36. Trombelli L, Heitz-Mayfield LJ, Needleman I, Moles D, Scabbia A (2002) A systematic review of graft materials and biological agents for periodontal intraosseous defects. J Clin Periodontol 29:117–135
37. Van Dijk LJ, Schakenraad JM, van der Voort HM, Herkströter FM, Busscher HJ (1991) Cell-seeding of periodontal ligament fibroblasts. A novel technique to create new attachment. A pilot study. J Clin Periodontol 18(3):196–199
38. Weltman R, Trejo PM, Morrison E, Caffesse R (1997) Assessment of guided tissue regeneration procedures in intrabony defects with bioabsorbable and non-resorbable barriers. J Periodontol 68:582–591