Efficacy of Combined Regenerative Treatments in Human Mandibular Class II Furcation Defects

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Background: The treatment of molar furcation defects remains a considerable challenge in clinical practice. The degree of success in the management of furcation involvement is highly variable and inversely related to initial probing depth (PD) measurements in these lesions. The identification of clinical measurements influential to the treatment outcomes is critical to optimize the results of surgical periodontal therapy. Therefore, the objective of this study was to evaluate the clinical response of mandibular buccal Class II furcation lesions to a combined regenerative treatment modality.

Methods: Sixty patients were divided into two (n = 30) treatment groups. An experimental combined regenerative therapy (ET) was compared to open flap debridement (OFD). The ET was a combination of a composite graft consisting of bioabsorbable hydroxyapatite and tetracycline (3:1), a guided tissue regeneration barrier, and a coronally advanced flap. The clinical variables evaluated were plaque, bleeding on probing, gingival recession, PD, vertical attachment level (VAL), horizontal attachment level (HAL), furcation vertical height, furcation horizontal depth, and the amount of tissue under the barrier membrane at uncovering. Reevaluation was performed 12 months after the surgical procedure.

Results: Both treatments resulted in improvements in all clinical variables evaluated. Postoperative measurements revealed a reduction in PD of 3.65 ± 0.6 mm and 0.60 ± 1.0 mm; VAL gains of 3.05 ± 0.6 mm and 0.65 ± 0.6 mm and HAL gains of 3.45 ± 1.3 mm and 0.55 ± 0.7 mm in the ET and OFD groups, respectively. In the ET group, significant positive correlations were found between baseline PD and PD reduction at 12 months, and the initial VAL correlated positively with PD reduction and HAL gain. The horizontal furcation depth and amount of tissue formed under the membrane at uncovering correlated positively with PD reduction and HAL and VAL gains. For the OFD group, the initial PD correlated positively with PD reduction and VAL and HAL gains and correlated negatively with recession. Initial VAL correlated positively with PD reductions and VAL and HAL gains. The initial HAL correlated negatively with recession at 12 months.

Conclusions: ET exhibited significantly better clinical results, with more PD reduction, HAL and VAL gains, and a higher frequency of furcation closure compared to OFD and showed promise as a regenerative treatment technique. The ability to predict a response to treatment based upon pretreatment parameters was not consistent between groups; thus, prediction of treatment outcomes based on pretreatment measurements should be carefully evaluated for each treatment modality. *J Periodontol 2009;80:1756-1764.*

KEY WORDS

Bone substitutes; durapatite; furcation defects; guided tissue regeneration; therapeutics; transplants.

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doi: 10.1902/jop.2009.080605

Regeneration of the lost attachment apparatus and a return to predisease architecture are the ultimate goals of periodontal therapy. Furcation lesions are an especially important therapeutic problem.[1] Furcations are generally less responsive to therapy than non-furcated areas and/or single-rooted teeth, as reflected by a limited gain in attachment levels, less reduction in probing depths (PDs), more frequent bleeding on probing (BOP), and higher microbiologic counts after conventional non-surgical and open flap instrumentation of furcation surfaces.[2,3] Moreover, a long-term study[4] has shown that molars with periodontitis involving furcation have a higher rate of periodontal breakdown and tooth loss after therapy than molars in which furcation is not involved in the mode of therapy employed. Nevertheless, furcation lesions are well-established and documented models for the clinical evaluation of regenerative techniques, especially in mandibular molars.[5] The potential clinical[6] and biologic[7] benefits of regenerative therapies for the treatment of furcation and intrabony[8] defects have been extensively reviewed, and the predictability of these techniques compared to other methods of treatment as well as the identification of factors associated with improved outcomes have been suggested as critical elements for future research in the field.[5] Thus, the objective of this study was to evaluate the clinical response of mandibular buccal Class II furcation lesions to a combined regenerative treatment modality.

MATERIALS AND METHODS

Study Population and Experimental Design

The study was designed as a randomized, prospective, parallel-arm, controlled clinical trial. It was conducted in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 2000, after approval by the institutional review board of Federal Fluminense University. Written informed consent was obtained from all subjects.

The inclusion criteria of the study included: adult subjects with clinically detectable mandibular buccal Class II furcation defects and ≥5 mm attachment loss, presenting an unremarkable general health according to a medical history and clinical judgment, and not taking any medications for ≥6 months before the beginning of the study. Subjects with aggressive forms of periodontitis, smokers, and subjects with significant systemic diseases (e.g., cancer, acquired immunodeficiency syndrome, and diabetes) were not included in the study. Subjects who quit smoking ≥1 year before the beginning of the study were considered non-smokers and, if the other inclusion criteria were met, were included in the present sample. Additional exclusion criteria included: mandibular Class III furcation defects, presence of apical radiolucency, and a previous lack of cooperation with the maintenance program or use of antibiotics during the 12 months before the study baseline measurements were recorded.

The study population consisted of subjects who had previously been treated non-surgically for advanced chronic periodontitis and were adherent to maintenance care ≥1 year before the beginning of the study. Treatment included scaling and root planing and plaque control measures. Baseline full-mouth plaque and bleeding scores were recorded. The sites included for study were isolated furcations that did not respond adequately after a comprehensive initial therapy phase. Sixty adult patients (26 males and 34 females; age range: 41 to 63 years; mean age: 48.3 years) meeting the inclusion criteria were treated between December 2002 and July 2006 at the periodontology clinic of Federal Fluminense University. Only one defect was selected from each patient and was randomly assigned, by the toss of a coin, to one of the two treatment modalities used: 1) control = open instrumentation of the furcation via a flap procedure (open flap debridement [OFD]); and 2) test = an experimental combined regenerative therapy (ET) consisting of a composite graft composed of bioabsorbable microgranular hydroxyapatite (HA)‡ mixed with tetracycline hydrochloride (TTC)§ in a 3:1 proportion. The graft was then covered with a non-resorbable, porous, pure polytetrafluoroethylene barrier membrane,‖ and the flap was coronally positioned over the membrane to achieve primary closure.

Clinical Data Collection

Clinical parameters were assessed, as previously described,[1] using a cemento-enamel junction (CEJ) or, when applicable, another defined landmark as a fixed reference point. All measurements were recorded using a periodontal probe¶ with a rubber stopper by a masked, trained, calibrated examiner (CMLM), who was unaware of the treatment provided, at baseline and 12 months later. Measurements were recorded to the higher 0.5 mm. A full-mouth plaque score (FMPS) was recorded dichotomously as the percentage of total surfaces (four sites per tooth).[1] BOP was also assessed dichotomously at the same sites, and a full-mouth bleeding score was calculated.[1] The point of maximum convexity of the marginal gingival contour was used as the reference for measurements of the gingival margin (GM). Gingival recession (REC) was measured as the distance from the CEJ to the GM. PD was measured as the distance from the GM to the bottom of the gingival sulcus. PD and REC were used to calculate the vertical

‡ Bionnovation, São Paulo, SP, Brazil.
§ EMS, São Paulo, SP, Brazil.
‖ Bionnovation.
¶ PCP-UNC 15, Hu-Friedy, Chicago, IL.

attachment level (VAL). The horizontal furcation depth was measured at the furcation fornix with the root eminences of the mesial and distal roots as the fixed reference, thus establishing the horizontal attachment level (HAL). Clinical measurements obtained at baseline (immediately before surgery) and at 12 months are reported.

After surgical debridement of the furcation, the following measurements were made at the treated sites: 1) furcation vertical height (FVH) (distance between the furcation roof and the bony base of the defect measured from the fornix to the deepest probable site); and 2) furcation horizontal depth (FHD) (using the mesial and distal root eminences as the fixed reference level). At membrane removal, the furcations were assessed for the amount of new tissue in the furcation, which was designated as open probing new attachment (OPNA),[9] by subtracting the measurements of FVH and FHD obtained at the time of membrane removal from those obtained at baseline, thereby obtaining the recordings of gains in OPNA at vertical directions (PVg) and horizontal directions (PHg). At the time of membrane removal, clinical furcation closure was defined as an FHD measurement ≤1 mm[10] at the level of the furcation roof. Twelve months after the initial surgery, the vertical and horizontal soft tissue measurements were repeated and recorded. A furcation was classified as clinically closed at the 12-month reevaluation if the HAL measurement was ≤2 mm[11] and there was no BOP at the site. All vertical clinical measurements were performed with a manual periodontal probe,# and horizontal clinical measurements were performed with a calibrated Nabers probe** by a single calibrated investigator (CMLM) unaware of the treatment provided.

Statistical Analyses

Baseline measurements were subjected to intergroup comparisons and analyzed by the unpaired *t* and two-way Mann-Whitney tests. Statistical significance was set at the 95% probability level ($P<0.05$).

Intragroup comparisons between the baseline and 12-month measurements were analyzed by the one-way Mann-Whitney test. Intergroup comparisons were analyzed by the two-way Mann-Whitney test. Statistical significance was set at the 95% probability level ($P<0.05$). The frequency of furcation closure was analyzed by the χ^2 test with significance set at the 95% probability level ($P<0.05$). Correlations among the measurements were analyzed via stepwise multivariate analysis for multiple comparisons. Pearson correlation coefficients were calculated. Statistical significance was set at the 95% probability level ($P<0.05$) for all the tests performed. Sample size was determined by power analysis, which indicated that, with a sample of 20 subjects, the study would have >90% power to detect a 1-mm difference in the primary outcome measures HAL and VAL between the two groups.

Flap Design

Intracrevicular incisions were made, and a mucoperiosteal flap, including at least one tooth ahead and another behind the tooth being treated, was elevated by blunt dissection. A vertical releasing incision was performed at the mesial aspect of the flap whenever necessary to obtain better access to the surgical area. The furcation defect was carefully debrided with surgical curets and ultrasonic instruments with special effort to remove all of the granulation tissue. Thorough root planing was performed with hand, rotary, and ultrasonic instruments until all root surfaces attained a hard glassy surface. A fine-grain bur was used to finish the roof of the furcation. Abnormal projections of enamel (cervical enamel projections or enamel pearls) nearby or in the furcation were removed whenever present. After instrumentation, the root surfaces were washed with saline solution to remove any remaining detached fragments from the defect and surgical field. For the OFD group, the flaps were repositioned and sutured with 5.0 sutures using an anchor suture technique.

The sites treated with ET were accessed using identical flap procedures and, in addition, received a composite graft composed of bioabsorbable HA and TTC (3:1) and a polytetrafluoroethylene (PTFE) barrier membrane extending ≥3 mm from the defects' margins and 2 or 3 mm coronally to the CEJ.[8] The barrier was firmly secured in place with a sling suture. The flap was split at its apical extent, allowing free coronal positioning over the barrier membrane, and was secured in place with primary closure using sling sutures.

Postoperative Treatment

The patients were prescribed systemic antibiotic therapy consisting of 200 mg doxycycline†† the day before surgery, followed by 100 mg daily until day 20 after surgery. No surgical dressing was used. The patients were instructed to continue their regular home hygiene care, except in the operated area, which was cleaned by means of gentle topical applications of chlorhexidine gluconate (0.2%) in saturated cotton swabs. Analgesics were prescribed on an individual basis.

The sutures were removed 1 week after surgery. The combined therapy-treated sites were reopened 4 weeks after the first surgery to remove the barrier membrane. The furcations were inspected for the formation of tissue, and OPNA[9] was recorded. Care was taken not to disturb the tissue. The flaps were

PCP-UNC 15, Hu-Friedy.
** PQ2N, Hu-Friedy.
†† Vibramycin, Pfizer, São Paulo, SP, Brazil.

coronally positioned, as described, to completely cover the furcation entrance and, therefore, any newly formed tissue.

Maintenance Schedule

After surgery, all patients were seen weekly during the first 3 months and biweekly for the next 3 months. Thereafter, the patients were seen once monthly for the last 6-month period of the study. Maintenance visits consisted of reinforcement of oral hygiene procedures and professional supragingival scaling and coronal polishing. Additional oral chemical plaque control was performed two times a day for 1 week every 3 months by means of mouthrinses with a solution of chlorhexidine gluconate 0.2%.

RESULTS

Wound healing was uneventful for all treated cases. Soft tissues healed within normal limits, and no significant visual differences were noted between the treatment groups. Data obtained at the baseline measurements are presented in Table 1. No statistically significant differences were observed between the groups for any of the baseline clinical parameters (Table 1). Measurements of the furcation horizontal (FHD) and vertical (FVH) dimensions obtained during the surgical procedure were not significantly different between the groups (Table 1). The treatment groups had equivalent characteristics before treatment.

Furcation closure was evaluated at the time of membrane removal and at the 1-year evaluation. At the time of membrane removal, 19 of 30 (63.3%) ET furcations were evaluated as clinically closed. At the 1-year evaluation, 18 of 30 (60%) ET furcations were evaluated as clinically closed, and no closure was observed in the OFD furcations. These differences were highly significant (χ^2 test; $P<0.001$).

The data obtained at the 12-month measurements are presented in Table 1. At 12 months, within-group analysis revealed that the treatment modalities resulted in improved clinical conditions compared to baseline (Table 1). Clinical measurements revealed lower FMPS, less BOP, reduced PD, and gains in VAL and HAL for both treatment modalities (Table 1). The plaque and bleeding scores were similar for the OFD and ET groups, albeit not statistically significant.

The ET demonstrated statistically significant improvements over baseline measurements for all clinical variables tested (Table 1). Compared to the control group (OFD), the ET groups demonstrated significantly more PD reduction and HAL and VAL gains (Table 1). Both groups exhibited similar degrees of REC at 12 months (Table 1).

Table 1.

Baseline and Post-Surgical Measurements of Furcation Lesions

Parameters	OFD	Clinical Variables	Intergroup
FMPS			
Baseline	10.74 ± 3.5	10.26 ± 3.2	NS
1 year	9.64 ± 4.3	7.74 ± 3.3	*
Difference	1.10 ± 3.8	2.52 ± 3.1	*
Intragroup	NS	NS	
BOP			
Baseline	11.60 ± 3.6	10.79 ± 3.5	NS
1 year	8.73 ± 4.5	8.33 ± 3.7	NS
Difference	2.87 ± 4.1	2.46 ± 3.6	
Intragroup	*	*	
PD			
Baseline	5.95 ± 1.3	6.36 ± 1.0	NS
1 year	5.3 ± 1.0	2.80 ± 1.6	*
Difference	0.6 ± 1.0	3.56 ± 0.6	*
Intragroup	*	*	
REC			
Baseline	0.70 ± 0.9	0.70 ± 0.7	NS
1 year	−1.17 ± 0.8	−1.25 ± 0.7	NS
Difference	−0.47 ± 0.8	−0.55 ± 0.7	NS
Intragroup	*	*	
VAL			
Baseline	6.65 ± 0.8	7.06 ± 0.7	NS
1 year	6.00 ± 0.6	4.01 ± 1.0	*
Difference	0.65 ± 0.6	3.05 ± 0.6	*
Intragroup	*	*	
HAL			
Baseline	6.10 ± 1.4	4.85 ± 0.9	NS
1 year	5.56 ± 0.8	2.40 ± 1.3	*
Difference	0.55 ± 0.7	3.45 ± 1.3	*
Intragroup	*	*	
FVH			
Baseline	4.30 ± 1.3	4.45 ± 1.0	NS
Removal	NA	1.45 ± 1.1	NA
Difference	NA	3.0 ± 1.0	NA
Intragroup	NA	*	NA
FHD			
Baseline	4.70 ± 1.4	4.80 ± 1.0	NS
Removal	NA	1.22 ± 0.9	NA
Difference	NA	3.58 ± 1.0	NA
Intragroup	NA	*	NA

Intragroup comparisons were obtained between baseline and 1-year evaluations, except for FVH and FHD, which were obtained at baseline for both groups and at membrane removal at ET sites. Intergroup comparisons were obtained either at baseline or 1-year evaluations.
NS = non-significant; NA = non-applicable.
* Statistically significant (P <0.05).

Table 2.

Correlations Among Clinical Measurements and Outcome Variables in Treated Furcations

Group/ Parameters	PDred	VALg	HALg	RECc	PVg	PHg
ET						
PD	R = 0.5141 *P* = 0.04	R = 0.4931 *P* = 0.06	R = 0.2946 *P* = 0.29	R = –0.4698 *P* = 0.08	R = 0.3963 *P* = 0.15	R = 0.1468 *P* = 0.60
VAL	R = 0.5413 *P* = 0.04	R = 0.4453 *P* = 0.14	R = 0.5251 *P* = 0.04	R = 0.3072 *P* = 0.25	R = 0.3721 *P* = 0.10	R = 0.3525 *P* = 0.24
HAL	R = 0.5517 *P* = 0.03	R = 0.4369 *P* = 0.10	R = 0.5923 *P* = 0.02	R = –0.3164 *P* = 0.25	R = 0.4814 *P* = 0.06	R = 0.3122 *P* = 0.25
REC	R = 0.0885 *P* = 0.75	R = 0.0825 *P* = 0.77	R = 0.1652 *P* = 0.55	R = 0.1652 *P* = 0.56	R = 0.2991 *P* = 0.28	R = 0.1191 *P* = 0.67
FVH	R = 0.4431 *P* = 0.09	R = 0.3609 *P* = 0.18	R = 0.3557 *P* = 0.19	R = –0.1466 *P* = 0.60	R = 0.4326 *P* = 0.11	R = 0.1365 *P* = 0.63
FHD	R = 0.6566 *P* = 0.007	R = 0.5620 *P* = 0.02	R = 0.6408 *P* = 0.01	R = 0.1133 *P* = 0.69	R = 0.6011 *P* = 0.02	R = 0.6390 *P* = 0.01
PVg	R = 0.8975 *P* <0.0001	R = 0.9489 *P* <0.0001	R = 0.9404 *P* <0.0001	R = 0.0000 *P* = 1.0		R = 9166 *P* <0.0001
PHg	R = 0.8589 *P* <0.0001	R = 0.8977 *P* <0.0001	R = 0.8857 *P* <0.0001	R = 0.1426 *P* = 0.61	R = 0.9024 *P* <0.0001	
OFD						
PD	R = 0.6904 *P* = 0.004	R = 0.6016 *P* = 0.01	R = 0.6783 *P* = 0.005	R = –0.5555 *P* = 0.03		
VAL	R = 0.6849 *P* = 0.005	R = 0.6746 *P* = 0.006	R = 0.6000 *P* = 0.02	R = –0.3766 *P* = 0.16		
HAL	R = –0.0455 *P* = 0.87	R = –0.1164 *P* = 0.68	R = –0.420 *P* = 0.88	R = –0.5392 *P* = 0.04		
REC	R = 0.0885 *P* = 0.75	R = 0.0825 *P* = 0.77	R = 0.1652 *P* = 0.55	R = 0.1652 *P* = 0.56		
FVH	R = 0.1156 *P* = 0.68	R = 0.2157 *P* = 0.44	R = 0.3553 *P* = 0.19	R = –0.4432 *P* = 0.09		
FHD	R = 0.0391 *P* = 0.89	R = 0.1540 *P* = 0.58	R = –0.2510 *P* = 0.37	R = 0.3882 *P* = 0.15		

Correlations among the measurements analyzed via step-wise multivariate analysis for multiple comparisons.
red = reduction; g = gain; R = Pearson correlation coefficient; c = change; P = probability level.

Correlations among all measurements are shown in Table 2. Measurements were moderately to highly correlated (P <0.005). In the ET group, baseline PD was significantly correlated with PD reduction at 12 months and exhibited a tendency for correlation with VAL gains. Initial values of both VAL and HAL correlated positively with PD reduction and HAL gain. The horizontal furcation depth correlated positively with PD reduction and HAL and VAL gains. The amount of tissue formed under the membrane at the time of removal was highly correlated with PD reduction and HAL and VAL gains.

Table 2 shows that, in the OFD group, the initial PD correlated positively with PD reduction and VAL and HAL gains and correlated negatively with recession. Initial VAL correlated positively with PD reductions and VAL and HAL gains. Initial HAL correlated negatively with the 12-month recession. No correlations were found among the trans-surgical defect configuration and clinical responses of control furcations at 12 months.

DISCUSSION

In this study, sixty patients were treated by conventional surgical (OFD) or regenerative (ET) techniques. The treatment protocol emphasized the principles of careful soft tissue handling, wound stability, and infection control. Controls were treated by OFD only, whereas in the experimental group, a combined therapy composed of a composite bone substitute graft, a non-absorbable barrier membrane, and a coronally positioned flap was used. The results demonstrated that the combined therapy resulted in significantly more PD reduction, HAL and VAL gains, and furcation closure rates than traditional open flap instrumentation. The magnitude of change of the clinical parameters evaluated was within the range previously reported in guided tissue regeneration (GTR) studies. Reductions in PD were similar to those obtained in studies evaluating the effects of GTR employing either non-absorbable[12-17] or bioabsorbable membranes alone[15-19] and membranes combined with bone replacement grafts (BRG).[13,16,18,20-22] Similar results for HAL[12,15-17,19-27] and VAL[12-22,24,28] gains were also previously reported for the treatment of furcations with barriers and BRG. Other studies, however, reported more PD reduction[23-26] and VAL[23-26] and HAL gains[15,23] than observed in the present study.

It is evident from the literature[12-28] that great heterogeneity exists for results of clinical trials evaluating regenerative therapies in human furcation defects. Thus, the identification of factors that could possibly

influence the outcome of therapy and serve as predictors of success is of interest. Pontoriero et al.[29] suggested that the amount of remaining periodontium, the dimension (size and shape) of the defects, and the post-surgical plaque-control program were important for the success of GTR therapy. In an attempt to identify possible influential characteristics in the treated sample, correlation analyses of clinical and defect measurements were performed. Our data regarding the amount of tissue formed under the membrane at the time of removal were the single clinical factor highly correlated with all three major outcome measurements: PD reduction and HAL and VAL gains. To the best of our knowledge, this correlation has not been established before for GTR-treated furcation lesions. Despite the availability of limited data correlating the amount of tissue formed in the membrane-protected regenerative space and attachment gain in deep infrabony osseous defects,[30] it would appear that this measure is the best predictor of surgical success.

In the test (ET) group, there was also a positive correlation between baseline probing parameters (PD and VAL) and PD reduction and HAL and VAL gains at 12 months, which was similar to previous reports that demonstrated correlations between 1) initial PD and post-surgical PD reduction and HAL and VAL gains,[12,14] and 2) initial attachment levels (VAL and HAL) with post-surgical PD reduction[14] in expanded PTFE–treated furcations. We also found a positive correlation between measures obtained after flap reflection such as HFD and post-surgical PD reduction and HAL and VAL gain at 12 months. This finding is in contrast with previous studies that established a negative correlation between measurements of FHD and FVH and post-surgical attachment gain[28] and furcation closure.[22,27] Moreover, parameters of furcation closure at membrane removal (PVg and PHg) via OPNA were correlated with positive clinical outcomes measured by PD reduction and HAL and VAL gains at 12 months. Moreover, plaque accumulation, gingival inflammation, membrane exposure, edema, and suppuration during healing were also previously associated with poor results in GTR-treated sites.[28] Differences in treatment protocols and evaluation methods are possible explanations for such discrepancies. Other clinical parameters, such as gingival thickness <1 mm,[31] root divergence at the crest of bone,[22] defect volume <13 μl,[15] and persistence of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*)-positive sites[12] have also been identified as negative factors influencing the outcome of GTR therapy in mandibular Class II furcation defects; however, the possible influences of these factors were not evaluated in the present study.

In the control (OFD) group, PD reduction and VAL and HAL gains at 12 months were associated with initial PD and initial VAL. Increases in recession at 12 months were negatively correlated with initial PD and initial HAL. Interestingly, in sharp contrast to the ET group, no correlation was found among FHD and FVD and the clinical response of control furcations at 12 months. Therefore, taken together, these findings suggest that the response of human mandibular buccal Class II furcation defects to specific treatment modalities (such as regenerative versus conventional surgical instrumentation) is influenced differently by presurgical clinical parameters and by furcation-defect morphology. Thus, careful case selection based on clinical and defect-based characteristics may optimize the appropriate selection of specific surgical procedures to enhance the treatment outcomes of these lesions. Unfortunately, the best predictor of a successful outcome is determined after the initial healing phase and does not help the clinician in the treatment planning of the case.

In the present study, a BRG material mixed with tetracycline was employed in conjunction with a barrier membrane. Despite the positive outcomes obtained with the combined therapy reported in the present study, the advantages and possible additive benefits of the combined use of allografts, autografts, and/or implants with GTR membranes in combined regenerative therapies are controversial. Uncontrolled clinical case series[22,26] reported significant improvement and complete defect-closure rates of human Class II furcations with the use of autografts, allografts, or composite grafts associated with GTR membranes. Controlled clinical studies[24,32] demonstrated greater attachment gain, a higher percentage of complete furcation fill, and more stability of results over time than when membranes were used alone. A number of controlled clinical studies[13,16,18,19,20,23,25] demonstrated that the combined use of BRG and GTR promoted a significantly greater gain of hard tissue into treated furcation areas than OFD, or when the membranes were used alone. However, other controlled studies[17,21] failed to show any advantage of the use of allografts combined with bioabsorbable or non-resorbable membranes for the treatment of furcations and infrabony defects, and the biologic rationale for the use of "filler" has been repeatedly challenged.[7] The present study tested the effects of a microparticulate HA as the graft material for bone replacement. The particle size of the bone substitute graft material is considered critical to the fate of the graft.[33-35] In general, a particle size between 300 and 500 μm is considered ideal for periodontal use,[34,35] whereas particles <250 μm in diameter are considered less conductive to bone formation[35] and inhibitory to

the local differentiation of bone[33] in orthotopic or heterotopic sites. The BRG tested in the present study is a synthetic HA synthesized by means of a mild precipitation technique yielding a powder with ultrafine particles varying between 10 and 70 μm in diameter. The claim of superiority of this material is based on its very small particle size that allows complete resorption precluding encapsulation by connective tissue, as occurs with other materials with particles of larger dimensions.[36] Moreover, specific positive biologic responses have also been reported for this class of biomaterials including: 1) effective mitogenic effects for cultured fibroblasts, with efficacy similar to platelet-derived growth factor;[37] 2) increased phagocytosis by human osteoblasts resulting in increased genetic transcription, protein biosynthesis, and increased metabolic activity;[38] and 3) inhibition of alkaline phosphatase activity, stimulation of total protein synthesis, and stimulation of DNA synthesis in cultured periodontal ligament fibroblasts.[39] Thus, this biomaterial appears to possess key stimulatory effects in cells important for periodontal regeneration, such as periodontal ligament cells and osteoblasts, in contrast with previous views in the literature.[34,35] Interestingly, bone substitute grafts that demonstrated significantly different behaviors in similar animal studies[33-36] performed in ortho- or heterotopic sites exhibited virtually identical clinical outcomes to each other in comparative human clinical trials.[40,41] The complex wound environment of a human periodontal defect before and after therapy should create other biologic variables that cannot be controlled in specific animal studies, therefore limiting the direct extrapolation of findings from these studies to clinical practice.

In the present cases, the microparticulate HA was employed together with TTC as a composite graft. Other studies have shown that the mixture of HA and TTC increased the bone formation in alveolar bone defects in monkeys[42] and periodontal defects in dogs.[43] In the treatment of localized aggressive periodontitis, Mabry et al.[44] and Evans et al.[45] demonstrated that this mixture was very successful in obtaining more bone filling in interproximal bone defects. Pepellasi et al.[46] demonstrated that a composite HA:TTC graft associated with coronally positioned flaps was also very successful in the treatment of mandibular furcation lesions, resulting in significantly more PD reduction, probing attachment level gain, and complete furcation fill than controls using a modified Widman flap. These studies suggest that an HA:TTC composite graft can be beneficial for the treatment of periodontal lesions. Santana and Van Dyke[1] reported that the combined use of a composite HA:TTC graft in conjunction with a non-absorbable barrier was more effective than open debridement in the treatment of maxillary furcations. All of these studies are in agreement with the findings of the present report, demonstrating the superiority of the combination tested in the regenerative therapy of periodontal bone lesions and suggesting that these biomaterials have the potential to be further explored therapeutically. Due to the limitations imposed by the materials and methods of the present study, the relative importance of the composite graft and barrier membrane in the clinical outcomes recorded could not be evaluated. Further studies comparing the results obtainable by each component of the ET are in progress to clarify this concern.

CONCLUSIONS

The treatment of human mandibular buccal Class II furcation defects with a combined regenerative technique resulted in significant improvement of the clinical parameters evaluated. However, the clinical response to specific treatment modalities (such as regenerative versus conventional surgical instrumentation) was influenced by presurgical clinical parameters and furcation defect morphology.

ACKNOWLEDGMENTS

No funding was received for this study. The authors report no conflicts of interest related to this study.

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Submitted November 23, 2008; accepted for publication June 23, 2009.