Effects of Vitamin-B Complex Supplementation on Periodontal Wound Healing

Rodrigo F. Neiva,* Khalaf Al-Shammari,[†] Francisco H. Nociti Jr.,[‡] Stephen Soehren,* and Hom-Lay Wang*

Background: Reports have demonstrated that nutrient supplements, in particular vitamin-B complex (Vit-B), can positively influence wound healing processes. However, limited information is available on the effects of Vit-B on periodontal wound healing.

Methods: A total of 30 patients (13 males, 17 females) presenting with generalized moderate to severe chronic periodontitis were enrolled in this study. All subjects presented ≥ two teeth in the same sextant with probing depth (PD) ≥5 mm and bleeding upon probing (BOP) in need of access flap surgery (AFS). This study was a randomized, double-masked, placebo-controlled clinical trial. Subjects were instructed to take one capsule a day of either Vit-B (50 mg of the following: thiamine HCl, riboflavin, niacinamide, d-calcium pantothenate, and pyridoxine HCl; 50 µg each of d-biotin and cyanocobalamin; and 400 mcg of folate) or placebo for 30 days following AFS. Clinical attachment levels (CAL) and N-benzoyl-dl-arginine-2naphthylamide (BANA) test scores were measured at baseline and at 90 and 180 days following surgical intervention. Assessments of the healing response were also performed using BOP, gingival index (GI), and plague index (Pl) at baseline and 7, 14, 30, 90, and 180 days. The mean results of each parameter were averaged within a group. Differences between groups were analyzed by using repeated measures analysis of variance (ANOVA).

Results: Both groups experienced comparable levels of PD reduction following AFS (test: -1.57 ± 0.34 ; control: -1.50 ± 0.21). Changes in mean CAL were more favorable in Vit-B supplemented subjects (test: $+0.41 \pm 0.12$; control: -0.52 ± 0.23 ; P=0.024). Stratified data demonstrated significantly better results for the test group in both shallow (test: -0.08 ± 0.03 ; control: -1.11 ± 0.27 ; P=0.032) and deep sites (test: $+1.69 \pm 0.31$; control: $+0.74 \pm 0.23$; P=0.037). No significant differences were observed between groups regarding PI, GI, and BOP. BANA test values were significantly reduced in both groups after surgical treatment and no significant differences were noted between groups.

Conclusion: Vitamin B-complex supplement in combination with AFS resulted in statistically significant superior CAL gains when compared to placebo. *J Periodontol* 2005;76:1084-1091.

KEY WORDS

Surgical flaps; vitamin-B complex; wound healing.

reatment modalities available in periodontics can be broadly classified into either surgical or non-surgical approaches. Response to both treatment modalities varies and a number of factors have been suggested to play a role in the healing response after periodontal therapy. 1 Periodontal research has attempted to understand and modify the factors that interfere with or slow down periodontal wound repair or regeneration.²⁻⁵ The effects of complete daily nutrition on the periodontal wound healing process have not been extensively studied. This field has gained increased attention in recent years especially in the medical literature. 6-13 The current understanding of the effect of nutrition on periodontal disease and periodontal healing is mainly composed of hypotheses of the positive effects that either a well-balanced nutrition or supplementation of nutrients could affect the outcome of periodontal therapy. 14 Deficiencies of specific nutrients are fairly well known, but only a few studies observed the effects of nutrient supplementation in treated patients. Positive results have been shown in some studies, encouraging further research in this field. 15,16

Among all nutrients necessary for normal physiological functions, the vitamin-B complex may be important for periodontal wound healing.¹⁷ Thiamin, niacin, riboflavin, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, and

^{*} Department of Periodontics/Prevention/Geriatrics, School of Dentistry, University of Michigan, Ann Arbor, MI.

[†] Ministry of Health, Kuwait.

[†] Department of Periodontics, School of Dentistry, University of Campinas, Piracicaba, SP, Brazil.

biotin together form the water-soluble vitamin-B complex. Thiamin, also known as vitamin B₁, is required for the normal function of muscles and nerves because it converts glucose to energy. 18 Niacin, known as vitamin B₃, plays a role mainly in helping enzymes to function properly. 19 Riboflavin, also known as vitamin B₂, has been proven to be essential to normal growth, muscle development, and hair coat.²⁰ Pantothenic acid enables the body to create usable energy from carbohydrates, fats, and proteins. 21 Vitamin $B_{\underline{6}}$ is used by the body in the utilization of amino acids. ²² Folic acid and vitamin B₁₂ (also called cyanocobalamin) are two closely related B complex vitamins that are necessary for the bone marrow to produce red blood cells, and a deficiency of either can lead to advanced anemia.²³ Biotin is one of the most discussed of all vitamins, primarily because of its role in collagen synthesis. It is generally necessary for growth, digestion, and muscle function.²⁴ The vitamin-B complex group has also been reported to positively influence other wound healing processes.^{25,26}

This study aimed to further explore this field of research by examining the effects of vitamin-B complex supplementation following periodontal access flap surgery (AFS) on periodontal clinical and microbial parameters.

MATERIALS AND METHODS

Study Population

A total of 30 patients with generalized moderate to advanced chronic periodontitis and in need of at least one quadrant of periodontal surgery were enrolled in this study from the patient population at the University of Michigan, School of Dentistry. All patients had to present with two or more teeth in one quadrant with probing depth (PD) ≥5 mm with bleeding on probing (BOP) and were scheduled for access flap surgery by the examiner. A total of 1,044 tooth sites (504 test; 540 control) were treated. All patients must have completed initial periodontal therapy (scaling and root planing [SRP]) prior to inclusion in the study and demonstrated adequate oral hygiene levels (plaque score ≤20%). Half of these subjects (seven males, eight females; 10 smokers) were randomized to the test group (vitamin-B complex), and the other half (six men, nine women; seven smokers) to the control group (placebo). The mean age observed in the test group was 46.2 years (range, 39 to 62) and 47.9 years (range, 38 to 65) in the control group (Tables 1 and 2).

Study Design

The use of human subjects in this study was revised and approved by the Health Sciences Institutional Review Board of the University of Michigan. This study was a randomized, double-masked, vehicle-controlled clinical trial. Randomization was performed by placing 15 red (controls) and 15 green (test) marbles in a bag,

Table I.

Demographics

	Test	Control
Males/females	7/8	6/9
Mean age, years (range)	46.2 (38-65)	47.9 (39-62)
Smokers/non-smokers	10/5	7/8

Table 2.

Distribution of Sites

	Tooth Type		PD Category			
Group	Single- Rooted		Shallow	Moderate	Deep	Total
Placebo	396	144	163	231	146	540
Vit-B	288	216	129	249	126	504

from which the patient selected one. Vitamin-B complex supplement capsules, consisting of 50 mg of the following: B_1 (thiamine HCl), B_2 (riboflavin), B_3 (niacinamide), B₅ (d-calcium pantothenate), B₆ (pyridoxine HCl); 50 mcg (μ g) each of B₇ (d-biotin) and B₁₂ (cyanocobalamin); 400 mcg of vitamin B_C (folic acid), was given to the test group while placebo capsules were administered to controls. Two calibrated dental examiners recorded all measurements. The dental examiners remained masked to the study treatments of all patients throughout the study period. Calibration trials were performed prior and during the study to ensure repeatable intra- and interexaminer reliability. Mean prestudy reproducibility of measurements detected was 88.4% and mean intrastudy reproducibility reached 92.3%. All patients received AFS performed in the study quadrant by the same surgeon (RN). Patients were given a bottle containing 50 test (vitamin B-complex) or control (placebo) capsules immediately after surgery and instructed to take one capsule each morning until the 30-day postoperative appointment. Postoperative analgesia was provided through the prescription of acetaminophen/hydrocodone analgesics. Non-steroidal anti-inflammatory analgesics were not used to avoid potential anti-inflammatory effects which might influence the healing outcome measures. Postoperative appointments were performed at 7, 14, and 30 days after surgery.

Oral Hygiene and Supportive Treatment

Patients were instructed to avoid using mechanical plaque control on the surgical site for the first 7 days after surgery and to resume brushing at 7 days, but to

avoid proximity with the gingival margin. At 14 days, patients resumed normal brushing. Interdental cleaning aids were resumed 30 days after surgery. Periodontal maintenance appointments which included a review of medical and dental histories, supra- and subgingival instrumentation, and oral hygiene instructions and motivation were performed at both 90 and 180 days.

Table 3.
PI, GI, and BOP Mean Values

Parameter	Group	Baseline	7 Days	14 Days	30 Days	90 Days	180 Days
PI	Vit-B Placebo		2.20 ± 0.22 2.23 ± 0.14	—			
Gl	Vit-B Placebo		1.56 ± 0.15 1.68 ± 0.16				
ВОР	Vit-B Placebo	53 ± 13% 52 ± 10%	57 ± 13% 75 ± 25%	41 ± 19% 61 ± 27%	23 ± 14% 29 ± 14%	16 ± 6% 19 ± 8%	13 ± 6% 25 ± 11%

Outcome Measures

The clinical parameters evaluated in this study included CAL, measured from the cemento-enamel junction or a fixed reference point to the depth of the periodontal pocket; PD, measured from the gingival margin to the probeable base of the periodontal pocket; and BOP, recorded at each site as present or absent. In addition, the gingival index (GI) and plaque index (PI) scores were evaluated.²⁷ CAL was used to estimate the actual gains of periodontal attachment levels.^{28,29} GI was used to record the degree of soft tissue inflammation during soft tissue healing. PI was used to monitor improvements on patient's performed oral hygiene and GI.²⁷ All measurements were performed using a University of North Carolina probe at six sites per tooth. Only teeth receiving surgery were measured. Clinical parameters were evaluated at baseline and at 90 and 180 days following surgical care by two masked and calibrated examiners. GI and PI scores were also evaluated at 7, 14, and 30 days.

Microbial Assessment

The influence of vitamin B-complex supplementation during healing and tissue maturation was evaluated through microbiological assessments (BANA)§ at baseline and 90 and 180 days. The three sites showing higher PD values (≥ 5 mm) in the quadrant were selected for sampling. The same sites were reassessed at 90 and 180 days. After interpretation of the results, negative (0), weak positive (1), and strong positive (2) scores were given to each site. 30,31

Compliance

Compliance with either test or control capsules was evaluated by asking each subject to return the bottle containing the capsules at the 30-day appointment. The number of remaining capsules was subtracted from the original 50 supplied to determine how many were taken.

Statistical Analyzes

The data were analyzed on a per subject basis. Mean values for clinical parameters (PD, CAL, BOP, GI, PI)

and BANA test results were calculated for each subject and each site at all time points and averaged within a subject and then across subjects for the two groups. Differences between groups were sought using the repeated measures ANOVA. In addition, the data were aggregated based on baseline probing depth into three categories: 1) shallow, sites from 1 to 3 mm; 2) moderate, sites from 4 to 6 mm; and 3) deep, sites ≥6 mm. Mean values of PD and CAL were calculated for each category at all time points and averaged within each category and then across categories in the two groups. Differences between groups were analyzed using the repeated measures ANOVA.

RESULTS

Compliance

Compliance with either test or control capsules was evaluated using remaining capsule count. A high compliance rate was observed in both groups. The mean capsule intake in the test group was 30.3 capsules (range: 26 to 35) and 30.7 capsules (range: 28 to 33) in the control group. The compliance in the control group was 93.5% and 90.8% in the test group.

Plaque Index

Both groups began the study with comparable mean Pl values. A significant increase in the Pl values was observed at 7 days and 14 days after surgery. At 30 days, both groups demonstrated values comparable to baseline values. The greatest reduction was observed for both groups at 90 days, with a slight but insignificant increase observed at 180 days. No statistically significant differences in Pl were observed between groups at any time points (Table 3).

Gingival Index

Both groups began the study with similar mean GI values. A significant increase in the GI values was observed at 7 days, returning to values comparable to baseline at 14 days. At 30 days, both groups demonstrated a

§ BANA Test, BANAMet LLC, Ann Arbor, MI.

marked decrease, which continued to be observed at 90 days. At 180 days, the test group demonstrated a continued decrease of values, while the control group showed a slight rebound. However, no statistically significant differences were observed between groups at any time (Table 3).

Bleeding on Probing

Similar percentages of bleeding sites were observed at baseline. Since probing was not performed at 7, 14, and 30 days, BOP scores were determined by stimulation of the gingi-

val sulcus with a periodontal probe. A statistically significant increase was observed at 7 days for the control group, but not for the test group. The differences observed at 14 days post-surgery were also statistically significant. At both 30 and 90 days, both groups demonstrated a marked decrease in the percentage of bleeding sites with no statistically significant differences observed between groups. At 180 days, the test group demonstrated a continued decrease in percentage of bleeding sites, while the control group demonstrated a rebound in the number of bleeding sites. The differences observed at 180 days achieved statistical significance (P = 0.005) (Table 3).

Probing Depth and Clinical Attachment Level

Both groups initiated the study with similar mean PD values. These mean values were markedly decreased after periodontal surgical therapy at both 90 and 180 days. However, no statistically significant difference was observed between groups (Table 4). When data were analyzed based on smoking status, non-smokers showed lower PD reduction in the control group (2.7 \pm 0.39 mm versus 3.1 \pm 1.06 mm). However, no significant differences were noted between smokers and non-smokers in vitamin B-complex supplemented subjects (2.47 \pm 0.34 mm versus 2.52 \pm 0.25 mm).

Both groups also began the study with similar mean CAL values. The mean values appeared to slowly and constantly decrease after periodontal surgical therapy in the test group, while an increase was observed in the control group. A statistically significant difference was observed between test and control groups at 90 and 180 days. When the baseline mean CAL was compared with 180 day mean CAL, the control group showed a mean loss of CAL of 0.52 ± 0.23 mm, while the test group demonstrated a mean gain of 0.41 ± 0.12 mm. This difference between groups reached statistical significance (Table 4). Smoking status did not seem to affect CAL changes since both groups showed similar CAL changes for smokers and non-smokers (control: smokers 4.62 ± 0.45 mm, non-smokers 4.69 ± 0.23 mm; test: smokers 3.69 ± 0.22 mm, non-smokers $3.67 \pm$ 0.34 mm). Data were further categorized according to

Table 4.
PD and CAL (mm) Mean Values

Parameter	Group	Baseline	90 Days	180 Days	Change
PD	Vit-B Placebo	5.7 0 = 0.07	2.47 ± 0.16 2.50 ± 0.30	2 0.25	1.07 = 0.5 .
CAL	Vit-B Placebo	4.03 ± 0.94 4.07 ± 1.14	3.86 ± 0.80 4.33 ± 1.22	3.62 ± 0.82 4.59 ± 1.39	$+0.41 \pm 0.12$ -0.52 ± 0.23

Table 5.

PD Changes Based on Stratified Baseline PD

Site Depth	Baseline	90 Days	180 Days	Change
Shallow (1- Control Test	3 mm) 2.65 ± 0.47 2.67 ± 0.38			+1.02 ± 0.17*† -0.09 ± 0.02*
Moderate (Control Test	5.09 ± 0.78		3.01 ± 0.67*† 2.61 ± 0.59*†	
Deep (≥6 n Control Test	7.46 ± 1.24			-3.77 ± 0.52*† -4.53 ± 0.31*†

^{*} Statistical significance between groups.

baseline PD measurements into: shallow sites (1 to 3 mm) that showed a mean PD of 2.65 ± 0.47 mm for the control group and 2.67 ± 0.38 mm for the test group; moderate sites (4 to 6 mm) that showed a mean baseline PD of 5.09 ± 0.78 mm for the control group and 4.94 ± 0.63 mm for the test group; and deep sites (\geq 6 mm) that showed a mean PD of 7.46 \pm 1.24 mm for the control group and 7.42 ± 1.32 for the test group. At 90 days, a significant decrease in these values was observed for both groups. Overall, the mean PD changes for the control and test groups were: 1) shallow sites: $+1.02 \pm 0.17$ mm versus -0.09 ± 0.02 mm; 2) moderate sites: -2.08 ± 0.22 mm versus -2.33 ± 0.10 mm; and 3) deep sites: -3.77 ± 0.52 mm versus -4.53 ± 0.31 mm. Statistical significance was observed between groups when mean PD reduction of shallow and deep sites was compared (Table 5). At 90 days, a significant decrease in these values was observed in both test and control groups in moderate and deep sites, while a slight increase was observed in shallow sites. This significant decrease in mean CAL values was also observed at 180 days. Different levels of CAL gains were observed when the data was stratified based upon baseline PD (Table 6).

[†] Statistical significance between baseline and observed time points.

Table 6.

CAL Changes Based on Stratified Baseline PD

Site Depth	Baseline	90 Days	180 Days	Changes
	3 mm) 2.77 ± 0.23 2.95 ± 0.12		5.00 = 0.57	-1.11 ± 0.27* [†] -0.08 ± 0.03*
	4-6 mm) 4.60 ± 0.53 4.86 ± 0.38		$4.83 \pm 0.76^{\dagger}$ $4.07 \pm 0.68^{\dagger}$	
Deep (≥6 n Control Test	6.94 ± 1.03	6.14 ± 0.92*† 5.68 ± 0.88*†		+0.74 ± 0.23* +1.69 ± 0.31* [†]

- * Statistical significance between groups.
- † Statistical significance between baseline and observed time points.

BANA Test

Both groups demonstrated a significant reduction of positive tests (control: 1.22 ± 0.09 ; test: 1.23 ± 0.04) after surgical therapy, which was observed at both 90 days (control: 0.52 ± 0.03 ; test: 0.49 ± 0.11) and 180 (control: 0.83 ± 0.21 ; test: 0.44 ± 0.39) days. No statistically significant differences were observed between groups at any time.

DISCUSSION

Periodontal therapy aims to restore and maintain the periodontium in a state of health, function, and esthetics.³² Bacterial plaque and its byproducts in a susceptible host are considered to be the primary etiology of chronic periodontal disease.³³ In consequence, periodontal therapy begins with removal or modification of etiologic agents, such as bacterial plaque and calculus, allowing the periodontal tissues to heal. Response to treatment modalities varies and a number of factors have been suggested to play a role in the healing response after periodontal therapy. Longitudinal clinical trials have demonstrated that conventional periodontal surgical procedures can effectively reduce PD and promote CAL gains, especially when associated with improved levels of bacterial plague control and frequent professional maintenance care. 34,35

Among all nutrients necessary for normal physiological functions, the vitamin-B complex may be important for periodontal wound healing. This randomized clinical trial was designed to evaluate if the outcomes of periodontal surgical therapy could be enhanced by optimization of vitamin-B complex intake during the critical first 30 days of periodontal wound healing. Mean PD was significantly reduced after surgical therapy in both groups even though control sites had shown slightly higher PD values before treatment

(2.8 versus 2.4 mm). Nonetheless, no statistically significant differences were observed between groups. This may imply that the effects of vitamin-B complex supplementation may have been underestimated, possibly due to the resective nature of the surgical procedure applied. However, a positive influence of vitamin-B complex was observed when the data were analyzed in relation to smoking status. Research has demonstrated that response to either surgical or non-surgical periodontal therapy in smokers is compromised, when compared to non-smokers. 36-38 Subjects in the control group confirmed these findings since less PD reduction was observed among these patients. However, no differences were noted in regard to smoking status in the test group, demonstrating that vitamin-B complex supplementation might have compensated for the detrimental effects of smoking during wound healing.

To allow a more accurate and detailed analysis of these findings, the data were stratified based on baseline PD measurements, since different PD categories have shown to respond differently to periodontal treatment. 35,39-41 In the shallow sites (1 to 3 mm), the control group showed increased PD at 180 days (\pm 1.02 \pm 0.17 mm) from baseline values, while test sites remained unchanged (-0.09 ± 0.02 mm). For moderate (4 to 6 mm) and deep (≥6 mm) sites, significant PD reductions were found. The greatest PD reduction was noted in deep sites. Statistical significance was observed between groups when PD changes of shallow and deep sites were compared, showing more favorable outcomes for the vitamin-B complex group. This finding can be attributed to the optimal levels of biotin (vitamin B₇) intake during the first 30 days of the healing process. Biotin is considered a key element for collagen synthesis, which is an essential part of periodontal wound healing. Additionally, other members of the vitamin-B complex, such as thiamine, may have also enhanced wound repair as previously reported.^{25,42} Furthermore, vitamin B₅ has been shown to positively affect proline formation, a key element for collagen synthesis, and to promote cellular multiplication during initial wound healing. 26,43 This may have contributed to earlier cellular multiplication and flap reattachment, resulting in less granulation tissue formation and shallower PD after surgery. Positive effects have also been observed in the healing of repositioned flaps with vitamin B₃ supplementation, ⁶ suggesting that the vitamin presumably creates a more stable collagen structure during wound healing processes.⁴⁴⁻⁴⁶

These findings were also confirmed by CAL changes. The control group had a mean 0.52 ± 0.23 mm loss of CAL, while the test group showed a mean gain of 0.41 ± 0.12 mm. When stratified data were examined, shallow sites showed more CAL loss (-1.11 ± 0.27 mm) in the control group when compared to the test group (-0.08 ± 0.03 mm). No differences were noted between

groups in moderate sites. However, in deep PD sites the vitamin-B complex group showed significantly greater CAL gains when compared to the control group ($\pm 1.69 \pm 0.31$ mm versus $\pm 0.74 \pm 0.23$ mm, respectively). These findings are in agreement with previous reports that have demonstrated CAL gains after surgical treatment of periodontal pockets. $\pm 35.39 \pm 41.47.48$ Despite the similar levels of PD reduction noted between groups, CAL values appeared to improve throughout the study in the test group. It can be hypothesized that this group experienced less gingival recession following surgical therapy due to: 1) higher cellular multiplication; 2) enhanced collagen turnover; and 3) more resistant wound repair as a result of optimal vitamin B-complex intake.

The significant reduction of Pl at 90 and 180 days suggests that oral hygiene was significantly improved after surgical treatment. This improvement is attributed to the combined effects of elimination of retentive areas for bacterial plaque and utilization of interdental cleaning devices, and is in agreement with previous reports. 50-52 These findings were also positively correlated with GI throughout the study. No statistically significant differences of GI were observed between groups at any time, indicating that the positive effects of vitamin-B complex supplementation might have been masked by the nature of the surgical procedure. The significant reductions in GI values might be attributed to the overall change of the periodontal environment following surgical intervention, improvements in patient's self-performed oral hygiene, and reduced incidence of gingivitis. 53-57 The overall reduction in gingival inflammation was also confirmed by BOP values, which seemed to closely follow GI values throughout the study. These results suggest that vitamin B-complex supplementation reduces the clinical signs of inflammation (GI) and the incidence of BOP, even when comparable levels of plaque control were observed, and is in agreement with previous reports. 45,58-64

Microbiological assessment (BANA) demonstrated minimal differences between groups. Both groups demonstrated a significant reduction of baseline values after surgical therapy, which was observed at both 90 and 180 days. The BANA test focuses on identifying three periodontal pathogens, specifically Treponema denticola, Bacteroides forsythus, and Porphyromonas gingivalis. 65 In this study, samples were collected from sites with ≥5 mm PD at baseline with BOP. It is well understood that shallower periodontal pockets harbor significantly lower levels of periodontal pathogens than deeper pockets. 66-69 The significant reduction in BANA test values observed in both groups after periodontal surgical therapy may be attributed to the significant PD reduction. Since the deepest sites were selected for microbial sampling and the same sites sampled at 90 and 180 days, the PD reduction could have caused the increased rate of negative BANA test values.

All components of the vitamin B-complex are available in a well-balanced diet. Hence, additional intake of this group of vitamins throughout the study period was not controlled. While optimal levels of these vitamins were maintained during the first 30 days in the test group due to capsule ingestion, subjects in the control group might have also consumed optimal levels of these nutrients from their diet. In addition, vitamins are easily accessible to all patients, which might have encouraged subjects from both groups to continually utilize vitamin supplementation. Determination of serum levels of these vitamins at various times would certainly provide a better understanding of the effects of vitamin-B complex during periodontal wound healing.

A trend of the positive effects of vitamin-B complex supplementation on wound healing after periodontal surgery was observed in this study. Future studies including larger sample sizes and other forms of periodontal surgical procedures are certainly encouraged to further explore the effects of optimal nutritional intake among periodontal patients.

ACKNOWLEDGMENTS

The authors sincerely acknowledge Dr. George W. Taylor, Department of Cariology/Restorative Sciences/Endodontics, School of Dentistry, University of Michigan, Ann Arbor, Michigan, for his contribution during the development of this research project. This work was supported by the University of Michigan Periodontal Graduate Student Research Fund. The vitamins used in this study were kindly donated by TwinLab, Hauppauge, New York.

REFERENCES

- 1. Wikesjö J. Significance of early healing events on periodontal repair: A review. *J Periodontol* 1992;63:158-165.
- Giannobile WV, Lee CS, Tomala MP, Tejeda KM, Zhu Z. Platelet-derived growth factor (PDGF) gene delivery for application in periodontal tissue engineering. *J Periodon*tol 2001;72:815-823.
- 3. Froum SJ, Weinberg MA, Rosenberg E, Tarnow D. A comparative study utilizing open flap debridement with and without enamel matrix derivative in the treatment of periodontal intrabony defects: A 12-month re-entry study. *J Periodontol* 2001;72:25-34.
- 4. Caffesse RG, Quinones CR. Polypeptide growth factors and attachment proteins in periodontal wound healing and regeneration. *Periodontol 2000* 1993;1:69-79.
- 5. Caton J, Greenstein G. Factors related to periodontal regeneration. *Periodontology 2000* 1993;1:9-15.
- Collins TM, Denish A, Sheffield J, Mitra A, Stueber K, Smith YR. Nicotinamide enhances skin flap survival. Scand J Plast Reconstr Surg Hand Surg 1989;23:177-179.
- 7. Andrews M, Gallagher-Allred C. The role of zinc in wound healing. *Adv Wound Care* 1999;12:137-138.
- 8. Casey G. The importance of nutrition in wound healing. *Nurs Stand* 1998;13:51-54,56.

- 9. Partridge C. Influential factors in surgical wound healing. *J Wound Care* 1998;7:350-353.
- 10. Stadelmann WK, Digenis AG, Tobin GR. Impediments to wound healing. *Am J Surg* 1998;176:39S-47S.
- 11. Green S, McLaren S. Nutrition and wound healing. *Community Nurse* 1998;4:29-32.
- 12. Patten JA. Nutrition and wound healing. *J Gt Houst Dent Soc* 1998;69:14-15.
- 13. Rollins H. Nutrition and wound healing. *Nurs Stand* 1997;11:49-52.
- Neiva RF, Steigenga J, Al-Shammari KF, Wang HL. Effects of specific nutrients on periodontal disease onset, progression and treatment. *J Clin Periodontol* 2003;30: 579-589.
- Vogel RI, Lamster IB, Wechsler SA, Macedo B, Hartley LJ, Macedo JA. The effects of megadoses of ascorbic acid on PMN chemotaxis and experimental gingivitis. J Periodontol 1986;57:472-479.
- 16. George S, Bugwadia N. Nutrition and wound healing. *Medsurg Nurs* 1996;5:272-275.
- 17. Albina JE. Nutrition and wound healing. *J Parenter Enteral Nutr* 1994;18:367-376.
- Muroyama K, Murosaki S, Yamamoto Y, Ishijima A, Toh Y. Effects of intake of a mixture of thiamin, arginine, caffeine, and citric acid on adiposity in healthy subjects with high percent body fat. *Biosci Biotechnol Biochem* 2003;67:2325-2333.
- 19. Goldberg AC. A meta-analysis of randomized controlled studies on the effects of extended-release niacin in women. *Am J Cardiol* 2004;94:121-124.
- Kozik A, Korytowski W, Sarna T, Bloom AS. Interactions of flavins with melanin. Studies on equilibrium binding of riboflavin to dopa-melanin and some spectroscopic characteristics of flavin-melanin complex. *Biophys Chem* 1990;38:39-48.
- 21. Sonmez A, Lurie D, Chuong CJ. Effects of pantothenic acid on postoperative adhesion formation in a rat uterine horn model. *Arch Gynecol Obstet* 2000;263:164-167.
- 22. Morrow LE, Grimsley EW. Long-term diuretic therapy in hypertensive patients: Effects on serum homocysteine, vitamin B6, vitamin B12, and red blood cell folate concentrations. *South Med J* 1999;92:866-870.
- 23. Clements FW. Nutrition 7. Vitamin and mineral supplementation. *Med J Aust* 1975;1:595-599.
- 24. Baez-Saldana A, Zendejas-Ruiz I, Revilla-Monsalve C, et al. Effects of biotin on pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and markers for glucose and lipid homeostasis in type 2 diabetic patients and nondiabetic subjects. Am J Clin Nutr 2004; 79:238-243.
- 25. Alvarez OM, Gilbreath RL. Effect of dietary thiamine on intermolecular collagen cross-linking during wound repair: A mechanical and biochemical assessment. *J Trauma* 1982;22:20-24.
- 26. Aprahamian M, Dentinger A, Stock-Damge C, Kouassi JC, Grenier JF. Effects of supplemental pantothenic acid on wound healing: Experimental study in rabbit. *Am J Clin Nutr* 1985;41:578-589.
- 27. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol* 1967;38(Suppl.): 610-616.
- 28. Listgarten M. Periodontal terminology. *J Periodontol* 1993; 64:918-927.
- 29. Pihlstrom BL. Measurement of attachment level in clinical trials: Probing methods. *J Periodontol* 1992;63:1072-1077.

- 30. Kazor C, Taylor GW, Loesche WJ. The prevalence of BANA-hydrolyzing periodontopathic bacteria in smokers. *J Clin Periodontol* 1999;26:814-821.
- 31. Loesche WJ, Kazor CE, Taylor GW. The optimization of the BANA test as a screening instrument for gingivitis among subjects seeking dental treatment. *J Clin Periodontol* 1997;24:718-726.
- 32. American Academy of Periodontology. *Parameters of Care. J Periodontol* 2000;71(Suppl.):i-ii, 847-883.
- 33. American Academy of Periodontology. Guidelines for periodontal therapy (position paper). *J Periodontol* 2001; 72:1624-1628.
- Lindhe J, Westfelt E, Nyman S, Socransky S, Haffajee AD. Long-term effect of surgical/nonsurgical treatment of periodontal disease. J Clin Periodontol 1984;11:448-458.
- 35. Ramfjord S, Caffesse R, Morrison E, et al. Four modalities of periodontal treatment compared over 5 years. *J Clin Periodontol* 1987;14:445-452.
- 36. Preber H, Bergström J. Effect of cigarette smoking on periodontal healing following surgical therapy. *J Clin Periodontol* 1990;17:324-328.
- 37. Preber H, Bergström J. The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *J Clin Periodontol* 1986;13:319-323.
- 38. Preber H, Linder L, Bergström J. Periodontal healing and periopathogenic microflora in smokers and non-smokers. *J Clin Periodontol* 1995;22:946-952.
- Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK. Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. *J Periodontol* 1996;67:93-102.
- 40. Becker BE, Becker W, Caffesse RG, Kerry GJ, Ochsenbein C, Morrison EC. Three modalities of periodontal therapy: II. 5-year final results. *J Dent Res* 1990;69(Spec. Issue):219(Abstr. 882).
- 41. Lindhe J, Westfelt E, Nyman S, Socransky SS, Haffajee AD. Long-term effect of surgical/non-surgical treatment of periodontal disease. *J Clin Periodontol* 1984;11:448-458.
- 42. Alvarez OM, Gilbreath RL. Thiamine influence on collagen during the granulation of skin wounds. *J Surg Res* 1982;32:24-31.
- 43. Lacroix B, Didier E, Grenier JF. Role of pantothenic and ascorbic acid in wound healing processes: In vitro study on fibroblasts. *Int J Vitam Nutr Res* 1988;58:407-413.
- 44. Vaxman F, Chalkiadakis G, Olender S, et al. Improvement in the healing of colonic anastomoses by vitamin B5 and C supplements. Experimental study in the rabbit (in French). *Ann Chir* 1990;44:512-520.
- 45. Vaxman F, Olender S, Lambert A, et al. Effect of pantothenic acid and ascorbic acid supplementation on human skin wound healing process. A double-blind, prospective and randomized trial. Eur Surg Res 1995; 27:158-166.
- 46. Vaxman F, Olender S, Lambert A, Nisand G, Grenier JF. Can the wound healing process be improved by vitamin supplementation? Experimental study on humans. *Eur Surg Res* 1996;28:306-314.
- Lindhe J, Socransky SS, Nyman S, Westfelt E. Dimensional alteration of the periodontal tissues following therapy. *Int J Periodontics Restorative Dent* 1987;7(2):9-21.
- 48. Lindhe J, Nyman S, Westfelt E, Socransky SS, Haffajee A. Critical probing depths in periodontal therapy. *Compend Contin Educ Dent* 1982;3:421-430.
- 49. Graf W, Weiber S, Glimelius B, Jiborn H, Pahlman L, Zederfeldt B. Influence of 5-fluorouracil and folinic acid on colonic healing: An experimental study in the rat. *Br J Surg* 1992;79:825-828.

- 50. Rosling B. Periodontally treated dentitions: Their maintenance and prognosis. *Int Dent J* 1983;33:147-151.
- Froum SJ, Coran M, Thaller B, Kushner L, Scopp IW, Stahl SS. Periodontal healing following open debridement flap procedures. I. Clinical assessment of soft tissue and osseous repair. *J Periodontol* 1982;53:8-14.
- 52. Ramfjord SP. Maintenance care for treated periodontitis patients. *J Clin Periodontol* 1987;14:433-437.
- 53. Levy RM, Giannobile WV, Feres M, Haffajee AD, Smith C, Socransky SS. The short-term effect of apically repositioned flap surgery on the composition of the subgingival microbiota. *Int J Periodontics Restorative Dent* 1999;19:555-567.
- 54. Levy RM, Giannobile WV, Feres M, Haffajee AD, Smith C, Socransky SS. The effect of apically repositioned flap surgery on clinical parameters and the composition of the subgingival microbiota: 12-month data. *Int J Periodontics Restorative Dent* 2002;22:209-219.
- 55. Ismail AI, Morrison EC, Burt BA, Caffesse RG, Kavanagh MT. Natural history of periodontal disease in adults: Findings from the Tecumseh Periodontal Disease Study, 1959-87. *J Dent Res* 1990;69:430-435.
- 56. Jeffcoat MK. Prevention of periodontal diseases in adults: Strategies for the future. *Prev Med* 1994;23:704-708.
- 57. Ramfjord SP. Maintenance care and supportive periodontal therapy. *Quintessence Int* 1993;24:465-471.
- 58. Brown RS, Di Stanislao PT, Beaver WT, Bottomley WK. The administration of folic acid to institutionalized epileptic adults with phenytoin-induced gingival hyperplasia. A double-blind, randomized, placebo-controlled, parallel study. *Oral Surg Oral Med Oral Pathol* 1991;71:565-568.
- 59. Waerhaug J. Effect of C-avitaminosis on the supporting structures of the teeth. *J Periodontol* 1958;29:87-97.
- 60. Glickman I. Acute vitamin C deficiency and periodontal disease. II. The effects of acute vitamin C deficiency upon the response of periodontal tissues of the guinea pig to artificially induced inflammation. *J Dent Res* 1948;27:201-210.
- 61. Glickman I. Acute vitamin C deficiency and periodontal disease. I. The periodontal tissues of the guinea pig in acute vitamin C deficiency. *J Dent Res* 1948;27:9-23.
- 62. Brunson EG, Smith JF, Dixon R. Vitamin B12: An aid to oral mucous membrane healing. *Oral Surg Oral Med Oral Pathol* 1967;24:102-112.
- 63. Smith JF, Bell J. Supplementary treatment for oral tissue healing. *Dent Surv* 1971;47:27-28.

- 64. Weimann BI, Hermann D. Studies on wound healing: Effects of calcium D-pantothenate on the migration, proliferation and protein synthesis of human dermal fibroblasts in culture. *Int J Vitam Nutr Res* 1999;69:113-119.
- 65. Kazor C, Taylor GW, Loesche WJ. The prevalence of BANA-hydrolyzing periodontopathic bacteria in smokers. *J Clin Periodontol* 1999;26:814-821.
- 66. Edwardsson S, Bing M, Axtelius B, Lindberg B, Soderfeldt B, Attstrom R. The microbiota of periodontal pockets with different depths in therapy-resistant periodontitis. *J Clin Periodontol* 1999;26:143-152.
- 67. Hitzig C, Fosse T, Charbit Y, Bitton C, Hannoun L. Effects of combined topical metronidazole and mechanical treatment on the subgingival flora in deep periodontal pockets in cuspids and bicuspids. *J Periodontol* 1997;68: 613-617.
- 68. Hellstrom MK, Ramberg P, Krok L, Lindhe J. The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *J Clin Periodontol* 1996;23:934-940.
- 69. Westfelt E. Rationale of mechanical plaque control. *J Clin Periodontol* 1996;23:263-267.

Correspondence: Dr. Rodrigo Neiva, Department of Periodontics/Prevention/Geriatrics, University of Michigan, School of Dentistry, 1011 N. University Ave., Room 3323B, Ann Arbor, MI 48109-1078. Fax: 734/763-5503; email: rneiva@umich.edu.

Accepted for publication November 2, 2004.