Subgingival Root Brushing in Deep Human Periodontal Pockets

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Abstract

Objective: The short-term clinical and microbiological effects of patient-applied subgingival root brushing were assessed on untreated deep human periodontal pockets. **Methods:** Assessments of plaque, bleeding on probing, probing depth, total cultivable subgingival counts, and cultivable counts and proportions of six putative periodontal pathogens were carried out at baseline and after 14 days on two contralateral $\geqslant$ 6 mm bleeding interproximal posterior sites in each of 11 adults with untreated chronic periodontitis. One of the sites was randomly assigned to daily patient-applied subgingival root brushing for 14 days, and the other to remain with the patient's pre-existing tooth brushing and flossing regimen. No other periodontal therapy was performed during the 14 test days. **Results:** Significant reductions in plaque, bleeding on probing, probing depth, total subgingival counts, and levels of putative periodontal pathogens were found after 14 days of subgingival root brushing. Subgingival root brushing nearly eliminated bleeding on probing at test sites, reduced probing depths by a mean of 1.8 mm, and reduced cultivable subgingival proportions of six evaluated putative periodontal pathogens from a cumulative total of 14.1% to 0.8%. In comparison, no significant clinical or microbiological changes were detected after 14 days where the patient's pre-existing oral hygiene regimen remained unaltered. **Conclusions:** Subgingival root brushing over 14 days, in properly trained patients, induced favorable clinical and microbiological changes in deep periodontal pockets $\geqslant$ 6 mm even in the absence of professional subgingival debridement.

Key words: root brushing, chronic periodontitis, subgingival microbiota, oral hygiene, plaque control

Introduction

Adequate control of dental plaque biofilm growth from regularly performed patient self-care procedures is considered essential for maintenance of periodontal health and long-term success in treatment of destructive periodontal diseases (Löe, 2000; van der Weijden and Slot, 2011). Efforts to improve personal dental plaque biofilm control often focus on use of powered toothbrushes (Clayton, 2008), therapeutic mouth rinses (Moran, 2008), interdental cleaning devices (van der Weijden and Slot, 2011), and behavioral modification strategies to improve patient compliance with oral hygiene instructions (Renz and Newton, 2009).

However, conventional home self-care procedures with toothbrushes, floss, and mouthrinses are flawed in that, even with excellent patient compliance, they primarily target microbial dental plaque biofilms in supragingival areas, and provide relatively poor or no access into subgingival tooth locations, where pathogenic dental plaque biofilms interface with the advancing front of periodontitis lesions (Hansen and Gjermo, 1971; Pitcher *et al.,* 1980; Waerhaug, 1981). Despite this limitation, carefully supervised supragingival plaque control programs without subgingival instrumentation may induce favorable alterations in the composition of the subgingival microbiota, and decrease recovery of several putative periodontopathic species, in periodontal sites with shallow (1-3 mm) and moderate (4-5 mm) probing depths when applied for extended time periods ranging from six months to several years (McNabb *et al.,* 1992; Dahlén *et al.,* 1992; Sato *et al.,* 1993; al-Yahfoufi *et al.;* 1995, Ximenez-Fyvie *et al.;* 2000, Haffajee *et al.,* 2001; Gomes *et al.,* 2008). In comparison, more variable results occur in deeper periodontal pockets subjected exclusively to strict supragingival plaque control, where some studies report marked subgingival reductions in periodontal pathogens and anaerobic bacterial counts (Smulow *et al.,* 1983; Hellström *et al.;* 1996, Gomes *et al.;* 2008), and others reveal only minimal changes in

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subgingival microbial composition (Listgarten *et al.,* 1978; Kho *et al.,* 1985; Müller *et al.,* 1986; Beltrami *et al.,* 1987; Loos *et al.,* 1988; Westfelt *et al.,* 1998).

Subgingival root brushing is a relatively unexplored patient-applied technique offering direct mechanical disruption of both supragingival and subgingival dental plaque biofilms in daily home self-care regimens (Page *et al.,* 1991). Initial studies indicate that following intensive patient training to ensure proper brush placement and directed motion, subgingival root brushing may be safely used by patients in subgingival interproximal areas, resulting in diminished total microbial counts in moderate-depth periodontal pockets (Page *et al.,* 1992). The clinical and microbiological effects of subgingival root brushing on deep periodontal pockets ⩾ 6 mm have not been reported to date.

The purpose of the present study was to evaluate over 14 days, in the absence of professional subgingival instrumentation, the effects of patient-applied subgingival root brushing on the clinical and microbiological status of untreated deep periodontal pockets.

Material and methods

Subjects

A total of 11 systemically healthy, non-smoking adults (7 male, 4 female; mean age = 41.2 years; age range = 30-65 years) with untreated generalized moderate to advanced chronic periodontitis were included in the present study and provided their informed consent. Persons with a history of rheumatic fever, diabetes mellitus, venereal disease, blood dyscrasias, anomalies of the immune system, or who had received antibiotic or immunosuppressive drug therapy within the previous six months were excluded, as were individuals with aggressive periodontitis, gingival pain, or acute periodontal conditions. All of the study subjects stated that they performed whole-mouth tooth brushing and interdental flossing on at least a daily basis. Approval for this study was provided by the Temple University Human Subjects Protections Institutional Review Board.

Self-care procedures

The study subjects initially received a baseline examination where an explanation of the etiology and management of periodontitis was provided, emphasizing the need for subgingival infection control. Two interproximal test sites per study subject, each located on contralateral posterior teeth and presenting with ⩾ 6 mm probing depths and bleeding on probing, were randomly assigned by a coin flip, after assessment of baseline clinical and microbiological parameters, to either subgingival root brushing (one site) or control (one site) self-care procedures for a 14-day time period prior to the start of any subgingival mechanical debridement procedures. All study sites were located on interproximal aspects of either premolar teeth or flat surfaces of molars not associated with furcation entrance openings.

Periodontal sites assigned to the test self-care procedure received a total of 14 days of patient-applied subgingival root brushing on a daily basis as previously described (Page *et al.,* 1991; 1992), using a small 4-tufted brush (Spacemaster®, Wisdom Oral Care, Ltd., Evanston, IL, USA; *Figure 1*). In brief, subgingival root brushing was performed by the patient by placing the brush edge onto the proximal tooth surface, and then slowly advancing the brush bristles into the interproximal and subgingival areas with a wiggling motion *(Figure 2)*. The bristles were to be kept straight during the procedure, with the patient instructed to equally "balance" the bristle pressure on the ends of the bristles. Patient training for subgingival root brushing included initial "hands on" instruction, viewing of a 13-minute educational videotape providing close-up intraoral views demonstrating the technique, and three individualized reinforcement sessions, each of 15 minutes duration, to verify proper patient performance of subgingival root brushing during the 14-day study period. The study subjects were instructed to use the subgingival root brushing procedures only on the assigned periodontal site during the 14-day study period.

Contralateral sites assigned to control self-care procedures, as well as the rest of the dentition, continued to receive the patient's pre-existing tooth brushing and flossing regimen, which was not altered over the 14-day study period. No professional subgingival debridement of dental plaque biofilms or calculus deposits was performed prior to or during the course of the 14-day test period. Subsequent to the completion of the 14-day test period, all of the study subjects received comprehensive periodontal therapy and maintenance care.

Clinical examinations

A single trained periodontist (LRP) carried out all clinical evaluations on the study subjects. At baseline and after 14 days, all study sites were assessed with the plaque index (Löe, 1967) on a 0 to 3 scale. A gingival bleeding on probing index (Polson *et al.,* 1995) was graded within 30 seconds of probe removal as follows: 0 = no bleeding, 1 = single bleeding point, or a fine line of blood observed, 2 = interdental triangle becomes filled, or 3 = profuse bleeding observed immediately after probing. Periodontal probing depths were measured to the nearest millimeter using a Michigan-O probe with color-coded Williams markings. The clinical examiner was blinded to baseline assessment data when performing the 14-day examinations.

Intra-examiner reproducibility for the single clinical examiner was assessed by kappa analysis (Hunt, 1986) of blinded replicate probing depth

Table 1. Mean values SE of clinical and microbiological parameters at baseline and after 14 days with and without patient-applied subgingival root brushing.

Parameter and oral hygiene procedure group	Baseline Examination	Day 14 Examination
Plaque index		
root brushing	2.1 ± 0.2	0.1 ± 0.1**
control	2.3 ± 0.2	2.0 ± 0.3††
Gingival bleeding on probing		
root brushing	2.5 ± 0.2	0.5 ± 0.2**
control	2.5 ± 0.2	2.4 ± 0.3††
Probing depth (mm)		
root brushing	8.3 ± 0.4	6.5 ± 0.3
control	8.4 ± 0.5	8.3 ± 0.4†
% cultivable putative periodontal pathogens CFU/mL (log)[a]		
root brushing	14.1 ± 5.6	0.8 ± 0.5**
control	18.5 ± 7.4	23.0 ± 8.9†
Total cultivable periodontal pathogens CFU/mL (log)[a]		
root brushing	6.5 ± 0.3	2.2 ± 0.8**
control	6.6 ± 0.2	5.9 ± 0.6†
Total cultivable anaerobic organisms CFU/mL (log)		
root brushing	7.7 ± 0.3	7.0 ± 0.3*
control	7.6 ± 0.2	7.5 ± 0.1

[a]Cumulative data for *P. gingivalis, P. intermedia/nigrescens, F. nucleatum, P. micra, C. rectus,* and *A. actinomycetemcomitans.* Paired t-test for clinical parameters and Wilcoxon signed ranks test for microbiological parameters were used to test for significant differences between baseline and 14 day examinations; *$p < 0.01$, **$p < 0.001$. One-way ANOVA was used to analyze differences between root brushing and control groups at the same examination time point; †$p < 0.05$, ‡‡$p < 0.001$.

measurements taken 30 minutes apart on 234 periodontal sites in six subjects exhibiting a range of periodontal health and advanced periodontitis. Replicate probing depth measurements showed 97.9% agreement within 1 mm, and a kappa coefficient within 1 mm of 0.97. This probing depth kappa statistic value, which represents the proportion of agreement attained beyond chance, exceeds the kappa > 0.75 threshold level indicative of excellent examiner agreement (Hunt, 1986).

Microbial examinations

The occurrence and proportional recovery of six putative periodontal pathogenic species, as well as total viable anaerobic counts, were assessed by quantitative microbial culture in test and control sites at baseline and after 14 days. The putative periodontal pathogens presumptively identified in this study were *Porphyromonas gingivalis, Prevotella intermedia/nigrescens, Fusobacterium nucleatum, Parvimonas micra, Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans.*

Microbial sampling

At baseline and after 14 days, paper point subgingival microbial specimens were collected from test and control sites in each study subject after isolation and drying of sites, and removal of supragingival deposits and dental plaque from the orifice of sampled periodontal pockets. The study subjects were instructed to refrain from self-care oral hygiene procedures the night prior and in the morning on day 14 to enhance cultivable microbial count recovery, and minimize the potential impact of self-care procedures performed shortly or immediately prior to microbial sampling. Three fine absorbent sterile paper points

Table 2. Subgingival prevalence and proportional recovery of selected microbial species in study sites of 11 adult periodontitis patients at baseline and after 14 days with and without patient-applied subgingival root brushing.

Organism and oral hygiene procedure group	Baseline Examination		Day 14 Examination	
	No. of culture-positive subjects	Mean SE % recovery in positive subjects	No. of culture-positive subjects	Mean SE % recovery in positive subjects
P. gingivalis				
root brushing	1	2.20	0	-
Control	1	4.80	3	9.4 ±7.8
P. intermedia/nigrescens				
root brushing	6	9.3 ± 5.0	3	0.02 ± 0.01
control	5	12.9± 10.5	7	17.6 ± 10.9
F. nucleatum				
root brushing	9	3.0 ± 0.8	4	1.9 ± 1.0
control	4	4.2 3.4	5	4.1 ± 1.6
P. micra				
root brushing	7	6.0 ± 1.5	3	0.3 ± 0.3
control	9	10.4 ± 3.3	6	8.4 ± 2.9
C. rectus				
root brushing	7	3.3±2.1	0	-
control	8	2.6 ± 1.0	8	3.1 1.2
A. actinomycetemcomitans				
root brushing	0	-	0	-
control	1	0.04	1	0.15

(Johnson & Johnson, Windsor, NJ, USA) were inserted into each study site for 10 seconds and pooled into a glass vial containing 6-8 small glass beads and 2.0 mL of anaerobically prepared and stored VMGA III transport medium (Möller, 1966), which possesses a high preservation capability for oral microorganisms during post-sampling transit to the laboratory (Möller, 1966; Dahlén *et al.,* 1993). The sample vials were maintained at room temperature and processed within 24 hours at the Oral Microbiology Testing Service (OMTS) Laboratory at Temple University School of Dentistry, which is licensed for high complexity bacteriological analysis by the Pennsylvania Department of Health. The OMTS Laboratory is also federally certified by the United States Department of Health and Human Services to be in compliance with Clinical Laboratory Improvement Amendments (CLIA)-mandated proficiency testing, quality control, patient test management, personnel requirements, and quality assurance standards required of clinical laboratories engaged in diagnostic testing of human specimens in the United States (Rauch & Nichols, 2007). All laboratory procedures were performed by personnel that were blinded to the clinical status and self-care procedure assignments for the study subjects and their sampled periodontal sites, as well as their inclusion in the present study.

Microbial culture and incubation

At the OMTS Laboratory, the specimen vials were warmed to 35ºC to liquefy the VMGA III transport medium, and sampled microorganisms were mechanically dispersed from the paper points with a Vortex mixer at the maximal setting for 45 seconds. Serial 10-fold dilutions of the dispersed bacteria were prepared in Möller's VMG I anaerobic dispersion solution, comprised of pre-reduced, anaerobically sterilized, 0.25% tryptose-0.25% thiotone E peptone-0.5% NaCl (Möller, 1966). Appropriate dilution aliquots were plated onto non-selective enriched Brucella blood agar (EBBA) primary isolation plates (Rams *et al.,* 1996, 2011), comprised of 4.3% Brucella agar supplemented with 0.3% bacto-agar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione, onto Hammond's selective *Campylobacter* medium (Rams *et al.,* 1993), and onto selective trypticase soy-bacitracin-vancomycin (TSBV) agar (Slots, 1987). EBBA and Hammond's selective medium plates were incubated at 35ºC for seven days in a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA) containing 85% N_2-10% H_2-5% CO_2, and TSBV plates were incubated at 35ºC for three days in air-5% CO_2.

Microbial identification

Total anaerobic viable counts of *P. gingivalis, P. intermedia/nigrescens, F. nucleatum,* and *P. micra* were identified on EBBA plates using a ring-light magnifying loupe and presumptive phenotypic methods and criteria previously described (Rams *et al.,* 1992; 1996; 2011). *C. rectus* was quantitated on Hammond's

Figure 1

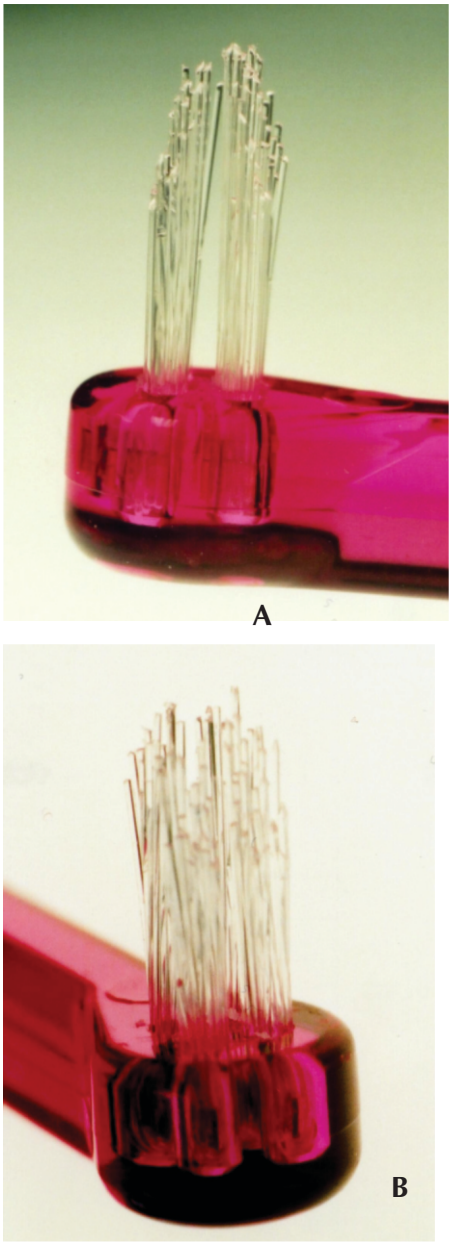


Figure 1. Lateral (A) and end (B) views of small 4-tufted brush used for subgingival root brushing.

medium, and *A. actinomycetemcomitans* on TSBV agar, as previously described (Rams *et al.,* 1993; Slots *et al.,* 1990). Proportional subject recovery of the evaluated putative periodontal pathogens was determined by comparing colony forming units (CFU) isolated on EBBA or selective media for each species with total cultivable anaerobic CFU counts on non-selective EBBA plates.

Data analysis

Frequency distributions and mean SE values for clinical and microbiological parameters of each oral hygiene self-care procedure group were calculated for baseline and 14-day data. Total CFU counts and cultivable proportions for putative periodontal pathogens at each examination point were determined by summing data for each study subject for the species *P. gingivalis, P. intermedia/nigrescens, F. nucleatum, P. micra, C. rectus,* and *A. actinomycetemcomitans*. Logarithmic (base 10) transformation of total cultivable CFU counts and putative periodontal pathogen CFU counts was carried out prior to statistical analysis to normalize data. A paired *t*-test was used to examine clinical parameters, and the nonparametric Wilcoxon matched pairs signed ranks test used to evaluate microbiological parameters, for statistically significant treatment group differences between baseline and 14-day mean values. Between-group comparisons at the baseline and 14-day examinations were made with one-way analysis of variance (ANOVA). A *p*-value of 0.05 was required for statistical significance. Data analysis was performed independent of the study's clinical examiner by author TER using the SAS 9.2 for Windows (SAS Institute, Inc., Cary, NC) statistical software package.

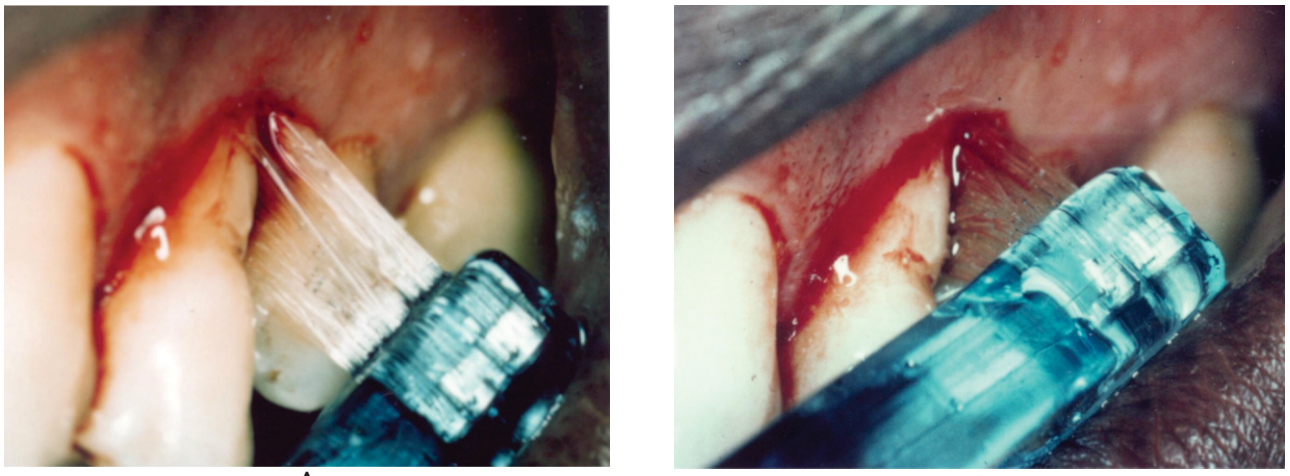
Results

Study sites assigned to each of the two self-care regimens did not exhibit statistically significant mean differences at baseline, with each yielding deep probing depths ⩾ 6 mm, bleeding on probing, supragingival plaque, and high subgingival counts and proportions of putative periodontal pathogens (*Table 1*).

At 14 days, periodontal sites subjected to daily patient-applied subgingival root brushing revealed statistically significant reductions from baseline in mean plaque index and gingival bleeding on probing index scores, probing depths, total subgingival cultivable counts, and total cumulative subgingival counts and proportions of the six evaluated putative periodontal pathogens (*Table 1*). Subgingival root brushing sites experienced a mean 1.8 mm reduction in probing depth, with supragingival plaque and gingival bleeding on probing reduced to almost negligible levels (*Table 1*). Subgingival proportions of the six monitored putative periodontal pathogens were reduced from 14.1% at baseline to 0.8% after 14 days of root brushing, and total subgingival counts of these species revealed an approximately 4-log reduction (*Table 1*). Among individual putative periodontal pathogens studied, *C. rectus* was undetectable at 14 days in seven initially positive periodontal sites exposed to subgingival root brushing, and marked decreases were seen in the prevalence and subgingival proportions of *P. intermedia/nigrescens* and *P. micra* (*Table 2*).

In comparison, all assessed clinical and microbiological parameters at control periodontal sites continuing to receive the patients' pre-existing tooth brushing and flossing regimen did not significantly differ from baseline to 14 days (*Table 1*). At 14 days, all of these parameters, except for total viable CFU/mL counts, were significantly greater than at periodontal

Figure 2



A **B**

Initial placement (A) and entry (B) of brush tips into an interproximal periodontal site.

sites assigned to subgingival root brushing (*Table 1*). No or minimal reductions from baseline among individual species of the putative periodontal pathogens monitored were found after 14 days with the patients' pre-existing tooth brushing and flossing regimen (*Table 2*).

No study subjects reported pain, or presented with gingival tissue trauma, periodontal abscess formation, or any other adverse clinical outcome in periodontal sites subjected to 14 days of subgingival root brushing or the patient's pre-existing tooth brushing and flossing regimen.

Discussion

The present study findings on deep periodontal pockets 6 mm confirm and extend initially reported observations on root brushing noted on moderate probing depths (Page *et al.,* 1991). Statistically significant reductions in plaque, bleeding on probing, probing depths, total subgingival counts, and proportional subgingival recovery of six evaluated putative periodontal pathogens were found on initially 6 mm probing depths (mean 8.3 mm) after 14 days of patient-applied subgingival root brushing in the absence of professional mechanical subgingival instrumentation. Interestingly, the extent of the probing depth reductions (mean 1.8 mm) and microbiological changes measured after 14 days of subgingival root brushing alone were similar in magnitude to those reported following professional subgingival scaling and root planing procedures on periodontitis sites with deep probing depths (Cobb, 2002; Petersilka *et al.,* 2002).

In comparison, similar contralateral control periodontitis sites assigned to the patients' pre-existing tooth brushing and flossing regimen showed no or minimal clinical and microbiological alterations from baseline values. The continued presence of high plaque index scores at control sites suggests poor patient performance with their pre-existing tooth brushing and flossing regimen. The relative absence of clinical or microbiological alterations on control sites also indicates that the microbial sampling technique used in the present study was unlikely to have exerted a notable therapeutic impact and contributed to the clinical and microbiological improvements measured on test sites subjected to subgingival root brushing,

The marked microbiological changes detected after 14 days of subgingival root brushing on deep periodontal pockets averaging over 8 mm in probing depth may in part be the result of improved supragingival plaque control, as reported in some (Smulow *et al.,* 1983; Hellström *et al.,* 1996; Gomes *et al.,* 2008), but not all, prior studies (Listgarten *et al.,* 1978; Kho *et al.,* 1985; Müller *et al.,* 1986; Beltrami *et al.,* 1987; Loos *et al.,* 1988; Westfelt *et al.,* 1998) on deep periodontal pockets. Instead, it is more likely that the patient-applied subgingival brushing delivered direct mechanical effects which disrupted crevicular plaque biofilms, reduced plaque biofilm microorganisms, and resulted in improved clinical parameters, similar to the report by Carey and Daly (2001), where a microbrush was professionally introduced into subgingival sites and rubbed onto the root surfaces of untreated periodontitis lesions. It is noteworthy that the marked clinical and microbiological improvements detected after 14 days of patient root brushing occurred without removal of subgingival calculus or endotoxin-exposed cementum from the periodontally affected teeth, similar to the post-systemic amoxicillin-metronidazole antibiotic therapy observations made by Lopez *et al.* (2006) on patients with severe periodontitis.

In previous studies where improved supragingival plaque control alone led to favorable changes in the composition of subgingival plaque biofilms in deep periodontal pockets (Smulow *et al.,* 1983; Hellström *et*

al., 1996; Gomes *et al.,* 2008), decreased total anaerobic counts were detected, as well as decreased occurrence and numbers of *P. gingivalis, P. micra, Dialister pneumosintes* and *A. actinomycetemcomitans,* but over longer study periods ranging from 21-180 days. Whereas the present study documented these types of subgingival microbiological changes occurring within a 14-day time period with subgingival root brushing, it is not clear that similar alterations occurred with improved supragingival plaque control alone in such a short time period, because the earliest microbiological re-evaluations in these favorable studies were performed at 30 days from baseline (Gomes *et al.,* 2008).

The present study has several limitations. Clinical periodontal attachment level was not measured, primarily due to the short time period (14 days) where follow-up clinical evaluations were performed, which is prior to the appearance of statistically significant clinical periodontal attachment gains that occur at periodontitis sites following disruption of subgingival plaque biofilms and decreases in gingival tissue inflammation (Proye *et al.,* 1982). However, probing depth reductions, which were assessed, provide a meaningful end-point outcome for judging the short-term efficacy of root brushing on periodontitis lesions (Paquette, 2005). The present study findings also do not address the histologic nature of gingival tissue healing associated with subgingival root brushing, the potential effects of subgingival root brushing combined with professional periodontal pocket debridement, nor the efficacy of subgingival root brushing in furcation involvements or on dental implant surfaces. Because *A. actinomycetemcomitans* was not isolated from test sites, the ability of subgingival root brushing to alter levels of this periodontal pathogen, capable of colonizing gingival connective tissues (Christersson *et al.,* 1987), is not known. However, *P. gingivalis,* another gingival tissue colonizing periodontal pathogen (Holt *et al.,* 1999), was reduced below cultivable detection in the single organism-positive study site after 14 days of subgingival root brushing. Real-time PCR may be better suited than microbial culture to detect low numbers of bacterial species in such situations (Gomes *et al.,* 2008), even though it does not differentiate between live and non-vital cells in subgingival specimens, and similar periodontal pathogen detection rates may be found with both real-time PCR and quantitative anaerobic culture (Boutaga *et al.,* 2003; 2006). The possibility also exists of examiner bias occurring in the measurement of clinical parameters in the present study, even though the clinical examiner was blinded to baseline assessment data when performing the 14-day clinical evaluations.

In clinical practice, subgingival root brushing is envisioned to be most frequently utilized as a self-care supplement, rather than a stand-alone substitute to professional periodontal debridement and conventional oral hygiene procedures on moderate to deep periodontal pockets. It is important to emphasize that effective home-based root brushing is dependent upon thorough and repeated training of patients in use of the technique on an individualized basis, and periodic hands-on reinforcement until the patient becomes skilled in the procedure. Additionally, specific brushes are required for proper performance of subgingival root brushing, such as an interproximal brush (Page *et al.,* 1991), an end-tufted brush (Page *et al.,* 1992), or the 4-tufted brush used in the present study.

Conclusions

Daily patient-performed subgingival root brushing without professional periodontal debridement over 14 days provided marked clinical improvements and favorable shifts in the composition of the subgingival plaque microbiota of untreated severe chronic periodontitis sites. Further study over longer post-treatment time periods to delineate variables critical to the success of subgingival root brushing in the management and prevention of periodontal infections in moderate to deep periodontal pockets appears to be warranted.

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