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Effect of Self-Performed Mechanical Plaque Control Frequency on Gingival Health in Subjects with a History of Periodontitis: A Randomized Clinical Trial

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ABSTRACT

Aim: This randomized clinical trial evaluated the effect of the frequency of self-performed mechanical plaque control (SPC) on gingival health in subjects with a history of periodontitis.

Materials and Methods: Forty-two subjects participating in a routine periodontal maintenance program were randomized to perform SPC at 12-, 24- or 48-hour intervals. Plaque index (PII) and gingival index (GI) were evaluated at baseline, and day 15, 30 and 90 of study. Probing depths, clinical attachment levels and bleeding on probing were assessed at baseline, day 30 and 90. Mixed linear models were used for the analysis and comparison of experimental groups.

Results: Mean GI at baseline remained unchanged throughout study (90 days) only in the 12-hour group ($0.7\pm0.1 vs. 0.8\pm0.1$; p<0.05). At the end of study, mean GI was significantly increased in the 48-hour group over that in the 12- and 24-hour groups. When GI=2 scores were considered, only the 48-hour group failed to maintain gingival health throughout the study (18.8%).

Conclusion: SPC performed at a 12- or 24-hour frequency appears sufficient to controlling gingival inflammation whereas this clinical status was not maintained using a 48-hour frequency in subjects with a history of periodontitis subject to a routine periodontal maintenance program (ClinicalTrials.gov: 50208115.9.0000.5346).

Keywords: dental plaque; gingivitis; oral hygiene; periodontal disease

Clinical Relevance

Scientific rationale for study: Self-performed mechanical plaque control is essential to maintain periodontal health. However, its frequency has not been elaborated in subjects with history of periodontitis.

Principal findings: Oral hygiene intervals of 12 and 24 hours appear compatible with maintained gingival health whereas an increase in gingival inflammation may be expected using a 48-hour interval.

Practical implications: Self-performed mechanical plaque control every 12 or 24 hours may suffice controlling gingival inflammation in subjects with a history of periodontitis subject to a routine periodontal maintenance program.

INTRODUCTION

Plaque-induced gingivitis is defined as an inflammatory lesion confined to the marginal gingival tissues resulting from a microbial dental plaque insult activating the local host immune-response. Gingivitis is reversible, reduced dental plaque exposure recaptures gingival health (Löe *et al.* 1965). Being the most prevalent form of periodontal disease (Gjermo *et al.* 2002; Susin *et al.* 2004, Tonetti *et al.* 2017), gingivitis may group into:

gingivitis within the intact periodontium, gingivitis within a reduced periodontium in a nonperiodontitis patient, and gingivitis within a reduced periodontium in a successfully treated periodontitis patient (Chapple *et al.* 2018). Epidemiology and natural history of gingivitis and periodontitis suggest that gingivitis precedes periodontitis (Lindhe *et al.* 1975, Löe *et al.* 1986), however not all cases of gingivitis advance into periodontitis (Brown & Löe 1993, Prayitno *et al.* 1993). Nevertheless, management of gingivitis appears a sound primary strategy in the prevention of periodontitis, and recurrent periodontitis (Chapple *et al.* 2015).

A principal strategy to gingivitis prevention is the establishment of effective oral hygiene routines including regular disruption of dental plaque through self-performed mechanical plaque control (SPC) using toothbrushes and supplementary interdental devices (Sambunjak *et al.* 2011). Various SPC frequencies have been proposed to sustain gingival health, for example, the American Dental Association recommends tooth brushing twice daily to prevent caries and gingivitis (ADA, 2019). Nevertheless, there is limited evidence whether that recommendation will suffice to sustain gingival health (Chapple *et al.* 2015). Studies from the 1970s indeed show oral hygiene intervals of 24 (Kelner *et al.* 1974) and 48 hours (Lang *et al.* 1973) compatible with gingival health. However, methodological limitations make it difficult to extrapolate these findings to a broader population.

Recent studies have shown personal oral hygiene performed at 12- or 24-hour intervals are compatible with gingival health (Pinto *et al.* 2013, de Freitas *et al.* 2016). SPC at 48-hour intervals appear insufficient to maintain gingival health levels, even when including use of toothpaste containing an antimicrobial agent (Pinto *et al.* 2013). However, these randomized controlled trials (RCTs) only include subjects without history of periodontitis. There is no evidence in the literature regarding the efficacy of SPC interval on gingival health in subjects with history of periodontitis participating in a periodic periodontal maintenance program. It thus appears important to establish SPC guidelines favoring gingival health also in subjects susceptible to periodontitis as it is known that sites consistently presenting with gingival bleeding represent an elevated risk for disease recurrence and in extension possible tooth loss (Schätzle *et al.* 2003, Lang *et al.* 2009). Moreover, persistent gingivitis provides conditions favoring *de novo* plaque formation (Ramberg *et al.* 1994, Ramberg *et al.* 2003), development of subgingival

plaque (Weidlich *et al.* 2001) and, in susceptible subjects, plaque presence will feed inflammation and, in turn, higher probability of disease activity (Lang *et al.* 1986). Indeed, a recent systematic review recommends that RCTs be undertaken to study these issues (Tonetti *et al.* 2015). The aim of the present RCT was to evaluate the effect of SPC frequency on maintenance of gingival health in subjects with a history of periodontitis.

MATERIALS AND METHODS

Study Design and Sample

This study was a single-masked, parallel design, three-arm RCT. Subjects diagnosed with periodontitis (Tonetti & Claffey 2005), treated at the Post-Graduation Clinic of the Federal University of Santa Maria (UFSM, Rio Grande do Sul, Brazil) and included in the clinic's periodontal maintenance program (recall interval 4-6 months), were eligible.

Subjects, 35 years or older, presenting with at least 12 teeth, Gingival index (GI) = 2 (Löe, 1967) and bleeding on probing (BoP) scores equal or less than 7.5% and 25% of the tooth surfaces, respectively, were offered to enter the study. Smokers, pregnant women, diabetics, subjects presenting with xerostomia, psychomotor disorders, with a fixed orthodontic appliance, or requiring antimicrobial prophylaxis to perform oral exams, and subjects requiring any medication known associated with gingival enlargement, or subjects having used antibiotic/anti-inflammatory drugs within 3 months of study were not included.

A sample of 14 subjects per group was estimated based on difference in mean GI of 0.25 and standard deviation of 0.21 (Pinto *et al.* 2013) considering a power of 80%; significance level of 0.05; and an attrition rate of 15%.

Ethical Considerations

Eligible subjects provided informed consent. This study was conducted by following the Guidelines and Norms Regulating Research involving humans. The research protocol was submitted and approved by the Research Ethics Committee, UFSM (CAAE: 50208115.9.0000.5346) and ClinicalTrials.gov (50208115.9.0000.5346). The study was conducted November 2015 through February 2018 at the UFSM.

Pre-Experimental Period

Prior to initiation of study, eligible subjects with a GI=2 greater than 7.5% received oral hygiene instructions until the reference value for inclusion in the study was reached. We arbitrarily established a percentage of gingival health and gingivitis as \leq 7.5% and \geq 15% of gingival bleeding (GI=2), respectively. The period between the subject's latest periodontal maintenance appointments and study initiation was approximately 3 months.

Randomization and Experimental Groups

Randomization was established using a computer program (Random Allocation Software, version 2.0) and maintained confidential using serially numbered opaque envelopes. Randomization was performed by investigator CSS not involved in data collection. Study subjects were randomized into three groups conducting SPC at either 12-, 24-, or 48-hour intervals constituting group 12h, 24h, and 48h, respectively. In addition, the subjects received a calendar containing the frequency and dates of SPC and a leaflet containing recommendations and contact information for the investigator responsible for the randomization to allow queries that may emerge.

Experimental Period

The study observation interval was 90 days. At baseline, study subjects were interviewed and received an oral prophylaxis using a rotating rubber cup (Microdont®, São Paulo, Brazil) and abrasive paste. Each subject received a kit containing a soft multi-tufted toothbrush (Colgate® Twister® Compact Head, New York, NY, USA), waxed dental floss (Colgate®, New York, NY, USA), and/or an interdental brush (Bitufo®, São Paulo, Brazil), dentifrice (Colgate® Maximum Protection Anticaries®, 90 g, New York, NY, USA), and a mouthwash containing 0.05% fluoride solution (Nova Derme, 1500 mL, Santa Maria, Brazil). Study subjects were instructed to use the mouthwash twice daily to provide oral comfort as a strategy to support compliance with the SPC protocol. That study subjects entering the trial with 7.5% or less sites with GI=2 is indicative of a high SPC standard regardless of brushing technique or supplementary interdental device.

Subjects were instructed to cover the width of their toothbrush at a single point with the dentifrice at SPC. Thus, approximately 0.5 g toothpaste would be used at each SPC event. The dentifrice tubes were weighed (Digital Balance Scale Professional-Mini, model 1480, Tanita Corporation, Tokyo, Japan) at the end of study to verify compliance

with the study protocol. Also, as a measure of compliance, questions regarding SPC frequency and possible reasons for non-compliance were offered. At the end of study, subjects were instructed to return to their usual and preferred SPC habits. Professional periodontal maintenance procedures were not performed during the study, however reestablished at the end of study.

Early Stopping Guideline

Subjects who showed 30% or more sites with gingival bleeding (GI=2) during the experimental period were removed from study. These subjects were instructed to resume their regular SPC and were reexamined weekly until restoration of gingival health, i.e., 7.5% or fewer sites showing gingival bleeding.

Clinical Parameters

Clinical recordings included the Plaque Index (PII; Silness & Löe 1964), the GI, probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BoP). PII and GI were recorded at baseline and day 15, 30 and 90 of study. PD, CAL and BoP were recorded at baseline and day 30 and 90. GI was estimated engaging approximately 1 to 2 mm of the gingival margin with the probe at a 45-degree angle with moderate axial pressure. PD was measured as the distance from the gingival margin to the most apical aspect of the sulcus/pocket. CAL was considered as the distance from the cemento-enamel junction to the most apical aspect of the sulcus/pocket. PD and CAL were measured in mm rounded to the closest whole mm. Interproximal recordings were performed as close as possible to the contact point. BoP was recorded up to 15 sec following PD recordings and classified as absent (0) or present (1). Clinical parameters were evaluated at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) using a periodontal probe (CP 15 UNC, Neumar / Brazil) and included all teeth except third molars.

Clinical examinations day 15, 30 and 90 were performed prior to SPC. Thus, "true" PII scores for each SPC interval were recorded. Examinations were performed by two masked examiners. Examiner APPR first evaluated PII whereupon examiner JM evaluated GI, PD, CAL, and BoP following SPC. Study subjects received a questionnaire concerning potential adverse events relating to the trial at examination day 30 and 90.

Examiner Training and Calibration

Study examiners received training from an experienced examiner (CHCM) consisting of a theoretical evaluation of periodontal parameters, discussion about each score or category and possible disagreements. Training was concluded as a reasonable level of consistency and understanding of the parameters were reached.

Examiner JM was calibrated before the start of the study for PD evaluation (Weighted Kappa=0.98) and CAL (Weighted Kappa=0.96). Intra-examiner reproducibility was evaluated in one thousand sites, through duplicate exams with an interval of 1 hour. Flat mouth mirror and periodontal probe (CP 15 UNC, Neumar, Brazil) were used in all examinations.

Statistical Analysis

Data were processed and analyzed using the Statistical Package for Social Science (SPSS for Windows, version 21.0, SPSS Inc., Chicago, IL, USA). Summary statistics included mean ± se PII, GI, PD, CAL, percentage sites with BoP and percentage sites with different GI scores. Data were analyzed by intention to treat and by protocol. Multiple imputation was used to replace missing data. Linear regressions were used to impute missing data, with time and group being used as predictors. Twenty imputations were run in order to better converge on strong approximations of missing data (Leech *et al.* 2015). Mixed linear models were used for the analysis and comparison of experimental groups. The best covariance structure (component symmetry) was tested. The parameters of the model were estimated through maximum probability. The level of significance was set at 5%. Subjects who reached the stopping rule had their data counted in subsequent examinations.

RESULTS

Forty-two subjects were randomized into the three SPC groups, four subjects did not complete the study (Figure 1). One subject (48h group) reached the early stopping guideline day 30 presenting with 30% of the sites with GI=2. This subject resumed regular SPC and was followed for 2 weeks regaining gingival health (\leq 7.5%, GI=2).

Table 1 shows baseline sociodemographic, behavioral, and clinical SPC group characteristics. No significant differences in mean PD, BoP and CAL were observed among groups.

Table 2 shows mean GI for the SPC groups at baseline, day 15, 30 and 90 analyzed by protocol. Identical observations were noted when intention to treat analysis was used (Appendix A). Only the 12h group maintained stable GI levels throughout the trial, the 24h group showed significant differences in GI levels day 30 and 90 compared with baseline. The 48h group showed increased GI levels throughout the trial including a significant difference from baseline to day 15. Significant differences in mean GI over the 90-day trial were observed between the 12h and 48h, and the 24h and 48h groups.

Figure 2 shows the percentage GI=2 in the different groups throughout the trial. The 48h group showed a higher percentage GI=2 day 90 compared with all other groups indicating a greater number of sites showing gingival bleeding compared with the 12h and 24h groups (p<0.05). When the percentage GI=2 at buccal/lingual and interproximal sites were analyzed, the same pattern emerged (Figure 3 & 4). GI scores for each experimental period are shown in Appendix B.

Baseline PII showed no significant differences between groups (Table 3). However, at the end of the 90-day experimental period, the 48h group showed an increased mean PII compared with the 12h and 24h groups. Intragroup observations showed a significant change in PII day 15 remaining stable through day 90 for the 12h and 24h groups. Subjects who performed SPC at 48h intervals experienced significant increases in PII day 15 and day 90.

Appendix B shows PD, CAL and BoP alterations by experimental period. Statistically significant intra- and intergroup PD and CAL alterations were not observed throughout the study.

Mean amount dentifrice used during the study was 92g, 50g and 55g for the 12h, 24h and 48h groups, respectively. Adverse events recorded day 30 and 90 suggested no remarkable differences between the 12-, 24- and 48-hour SPC protocols (Appendix C).

DISCUSSION

Recent studies explored the relationship between SPC frequencies and gingival health in subjects without a history of periodontitis (Pinto *et al.* 2013, de Freitas *et al.* 2016). Up to this time, evidence that explain the effect of SPC frequency on gingival health in subjects with a history of periodontitis, i.e. subjects proven susceptible to periodontitis, are lacking. This RCT demonstrated that subjects with a history of periodontitis subject to a routine periodontal maintenance program performing SPC at 12- or 24-hour intervals are able to maintain high levels of gingival health. Whereas subjects who performing SPC at 48-hour intervals demonstrate a significant decline in gingival health.

This study thus corroborates observations by Pinto *et al.* (2013) and de Freitas *et al.* (2016) finding 12- and 24-hour SPC intervals compatible with gingival health, although the study samples had distinct characteristics and differences in susceptibility to periodontitis. Lang *et al.* (1973) found that also a 48-hour interval compatible with maintained gingival health in contrast to the present study showing a significant deterioration in gingival health for subjects using a 48-hour SPC interval. The discrepancy between studies may be explained by methodological differences, whereas Lang *et al.* (1973) used a sample composed of dental students, and whereas SPC was performed under supervision, dental plaque stained and entirely removed at each oral hygiene event, the present study relied on regular patients from a periodontal maintenance pool, although with high oral hygiene standards.

There is no consensus in the literature relative to cutoff points to be considered determining clinically significant gingivitis. We arbitrarily established gingival health and gingivitis as 7.5% and 15% of the sites exhibiting gingival bleeding (GI=2), respectively. Within this standard, the 12h and 24h groups showed limited change in gingival condition (sites with GI=2 equaled 10.7% and 8.1%, respectively) throughout the study. However, the 48h group completed the study with a percentage of sites with GI=2 of 18.8%, showing approximately twice as much gingival inflammation in comparison. The same pattern was demonstrated when changes in the percentage GI=2 were analyzed for buccal/lingual and interproximal sites separately. Recently, a world workshop established a new classification for periodontal and peri-implant diseases, conditions defining periodontal health (Lang & Bartold 2018), and case-definition to plaque-induced gingivitis (Trombelli *et al.* 2018). Compared with proposed cut-off values, periodontal health was

maintained only in the 12h and 24h groups in the present study using GI=2 and no BoP as criteria to do define gingival inflammation,

Although the 12h and 24h group did not reach the cutoff for gingivitis in the present study and did not exhibit a significant difference in mean GI among them at the end of study day 90, both groups experienced an increase in gingival scores, the 12h group showing higher mean scores than the 24h group. This may be explained by a significant difference between the groups in mean GI at baseline, the 12h group entering the study with the highest mean GI (highest percentage sites with GI=1) and maintained this level throughout study whereas the 24h group experienced an increase in mean GI. From day 15 on there was no significant difference in mean GI between group 12h and 24h. The same realization may be applied for the absence of significant mean GI differences between the 12h and 48h group day 15 and 30.

Mean PII scores differed significantly at the end of study day 90 between subjects who performed SPC at 48-hour versus at 12/24-hour intervals revealing higher mean scores for the 48h group. Intra-group analyses showed an increase in mean PII day 15 for the 12h and 24h groups apparently from there on remaining stable through end of study. The 48h group experienced a significant increase in mean PII day 15 and again at the end of study. Likely, the 48h group higher plaque levels were associated with increase in gingival inflammation, persistent gingivitis shown to provide conditions favoring plaque formation (Ramberg *et al.* 1994, Ramberg, *et al.* 2003).

Limitations of the present study include difficulty to assess compliance with the study protocol, i.e., understand the frequency study subjects actually performed SPC. As a measure of compliance, at the end of study, dentifrice tubes were weighed revealing that the 48h group used more dentifrice than expected. This indicates that subjects in this group at scheduled SPCs either used more dentifrice than assigned, or performed SPCs at a higher frequency than randomized, or a compilation of both. However, this observation apparently did not afflict the results, as we observed distinct differences between the 12/24h groups and the 48h group, both regarding dental plaque accumulation and gingival health. An additional instrument to assess compliance was questioning at each examination event regarding compliance and possible reasons for non-compliance. There was no report of non-compliance that could bias assigned randomization. Another limitation of study is the strict criteria of $\leq 7.5\%$ of sites with GI=2

used as inclusion criteria. Thus, there is a reduction in the external validity of our findings, restricting the inference of our observations to subjects with a history of periodontitis with high standard oral hygiene measures.

In conclusion, SPC performed at a 12- or 24-hour frequency appears sufficient to control gingival inflammation whereas this clinical status was not maintained at a 48-hour frequency in subjects with a history of periodontitis subject to a routine periodontal maintenance program

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Table 1 – Sociodemographic, behavioral and clinical parameters by experimental group at baseline

Parameter			12h group	24h group	48h group (n=14)	р	
		(n=14)		(n=14)			
Age ($\overline{x} \pm sd$)* years			56.4±7.2	56.4±7.8	59.9±8.1	0.	
Gender n(%)#					0.	
Male			8(57.1)	4(28.6)	6(42.9)		
Femal	e		6(43.0)	10(71.5)	8(57.1)		
Socioeconomic I	evel n(%) [#]						
Family	<1 Brazilian mir	nimum wade	3(21 43)	2(14.28)	4(28 57)		
income	>2 Brazilian m	ninimum wage	J(∠1.43) 11/78 6)	2(14.20)	4(20.57) 10(71.5)	0.	
(monthly)			11(70.0)	12(05.7)	10(71.5)		
Education (years o	f study)	≤ 8 years	6 (43.0)	5(35.7)	7(50.0)	0	
Education (years o	i study)	> 8 years	8 (57.1)	9(64.3)	7(50.0)	0.74	
Behavioral n(%)#							
Frequency toothbr	ushing (daily)	≤ 2 times	2(14.3)	5(35.7)	3(21.4)	0	
		≥ 3 times	12(85.7)	9(64.3)	11(78.6)	Ŭ	
		Once weekly	-	1(7.2)	1(7.2)		
		2-3 times weekly	3(21.4)	1(7.2)	2(14.3)		
Frequency use inte	erdental	4-5 times weekly	1(7.2)	1(7.2)	-	0.68	
device		Daily	7(50.0)	8(57.1)	5(35.7)		
		2 times a day	2(14.3)	1(7.2)	1(7.2)		
		≥ 3 times a day	1(7.2)	2(14.3)	5(35.7)		
		Dental floss	2(14.3)	-	6(43.0)		
Interdental device (type)		Interdental brush	3(21.4)	2(14.3)	-	0.03	
		More than one	9(64.3)	12(85.7)	8(57.1)		
		device					
Clinics ($\overline{x} \pm sd$)*							
GI=2 (%)			5.0±1.9	4.2±2.3	5.5±2.3	0	
PD (mm)			2.4±0.2	2.33±0.3	2.3±0.3	0	
BoP (%)			13.5±3.5	14.0±4.5	13.1±4.3	0	
CAL (mm)			2.9±0.8	3.1±0.8	3.4±1.2	0.	

GI = Gingival Index; PD= Probing depth; BoP = Bleeding on probing; CAL = Clinical attachment level.

* ANOVA (Tukey post-hoc test)

chi-square statistics

		. , .	•		
			12h group	24h group	48h group
			(n=13)	(n=13)	(n=12)
		Baseline	0.7(0.1) ^{A,a}	0.5(0.1) ^{B,a}	0.6(0.1) ^{AB,a}
9	GI	15 days	0.8(0.1) ^{AB,a}	0.7(0.1) ^{A,ab}	0.9(0.1) ^{B,b}
		30 days	0.8(0.1) ^{AB,a}	0.7(0.1) ^{A,b}	1.0(0.1) ^{B,bc}
		90 days	0.9(0.1) ^{A,a}	0.8(0.1) ^{A,b}	1.1(0.1) ^{B,c}

Table 2 – Mean (±se) Gingival Index over the experimental period by group

Mixed linear models' analysis,

Different uppercase letters show intergroup differences (p<0.05)

Different lowercase letters show intragroup differences (p<0.05)

Table 3 – Mean (±se) Plaque Index over the experimental period by group

			12h group	24h group	48h group
			(n=13)	(n=13)	(n=12)
PII		Baseline	0.3(0.1) ^{A,a}	0.2(0.1) ^{A,a}	0.3(0.1) ^{A,a}
	15 days	0.5(0.1) ^{A,b}	0.4(0.1) ^{A,b}	0.8(0.1) ^{B,b}	
	30 days	0.5(0.1) ^{AB,b}	0.4(0.1) ^{A,b}	0.7(0.1) ^{B,b}	
		90 days	0.5(0.1) ^{A,b}	0.5(0.1) ^{A,b}	1.1(0.1) ^{B,c}

Mixed linear models' analysis

Different uppercase letters show intergroup differences (p<0.05)

Different lowercase letters show intragroup differences (p<0.05)

FIGURE LEGENDS

Figure 1: Study flowchart.

Figure 2: Percentage total sites with GI=2 by SPC interval over the experimental period.

Figure 3: Percentage buccal/lingual sites with GI=2 by SPC interval over the experimental period.

Figure 4: Percentage interproximal sites with GI=2 by SPC interval over the experimental period.







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