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Effect of sonic versus manual supervised toothbrushing on both clinical and biochemical profiles of patients with desquamative gingivitis associated with oral lichen planus: a randomized controlled trial.

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Running title: Sonic toothbrushing and DG

Abstract

Objectives: The aim of this randomized, parallel-design, clinical trial was to investigate the effectiveness of an intensive plaque control program with sonic versus manual toothbrushing on clinical outcomes and gingival crevicular fluid (GCF) levels of matrix metalloproteinases (MMP) in desquamative gingivitis (DG) patients.

Methods: A total of 32 patients affected by DG secondary to oral lichen planus (OLP) were consecutively recruited and randomly assigned to a test (n = 16) and control (n = 16) group. Both groups were enrolled in an intensive control program comprising supragingival scaling and polishing, and brush-specific instructions for a period of 8 weeks. The treatment of interest (test) was the use of a sonic-powered toothbrush and the standard treatment (control) was the utilization of a soft-bristle manual toothbrush for twice-daily home oral hygiene procedures. Periodontal parameters, patient-centred outcomes, MMP-1 and MMP-9 GCF levels were evaluated at baseline and 8 weeks after starting the program.

Results: The plaque control program resulted in statistically significant reduction in periodontal parameters with consequent improvement in the clinical features, painful symptoms and severity of DG lesions in both groups (all $P < 0.001$). When a sonic toothbrush was used there was a more significant decrease in clinical indices, mucosal disease scores, and GCF levels of MMP-1 and MMP-9.

Conclusions: This clinical trial reported the effectiveness of a combined protocol based on professional oral hygiene and supervised toothbrushing in OLP patients with DG. The daily use of a sonic toothbrush would seem to perform better in the short term.

Introduction

The presence of epithelial desquamation, erythema, and erosions on the gingival tissue is described as desquamative gingivitis (DG). Several autoimmune/mucocutaneous disorders can manifest as DG, although erosive, ulcerative and atrophic forms of oral lichen planus (OLP) are the most common causes.[1-5] Gingival manifestations could differ in extent and severity, from mild localized lesions to more severe areas with spontaneous hemorrhage.[2] While the milder presentations may include sensitivity to spicy or acidic foods, or discomfort with particular dentifrices, the more severe presentations are likely to be symptomatic and have significant impact on patient's quality of life.[5,6] The painful gingival and oral lesions can discourage patients from brushing effectively and may indirectly increase the long-term risk for periodontal tissue breakdown via plaque accumulation at specific sites.[7,8]

Recently, Ertugrul and coworkers hypothesized that the increased levels of active matrix metalloproteinases (MMP)-1 and MMP-9 in the gingival tissue and gingival crevicular fluid (GCF) of OLP patients in combination with poor oral hygiene may cause an increase in tissue breakdown in the long-term.[9] Collagen, the major component of the extracellular matrix in periodontal tissue, appears to be the main target in the degradation process in both OLP and inflammatory periodontal disease.[10] MMP-1 is a key regulator of connective tissue remodeling and exists in high concentrations in inflamed gingiva[11], while MMP-9 acts on denatured collagen, in particular the collagen type IV.[12] MMP-9 positive cells were found in the epithelium in OLP lesions and are capable of disrupting the epithelial basement membrane with consequent impairment of gingiva defense mechanisms.[13,14]

In the management of DG lesions, the current recommended clinical pathways suggest that initial treatment should also focus upon controlling oral hygiene and avoiding precipitating factors.[15,16] Although a meticulous plaque removal does not bring about complete resolution, a structured control could be effective in improving the oral health-related quality of life and clinically observed gingival lesions.[8]

Up to now, data concerning home oral care in OLP patients are scarce.[17-20] Only one study analyzed the clinical effectiveness of powered toothbrushing in motivated OLP patients compared to normal plaque control regimen without any additional advice.[21] Sonic toothbrush generates high-amplitude and high-frequency bristle motion, creating a gentle dynamic cleaning action that drives fluid forces along the gum-line and is capable of dislodging dental plaque in hard-to-reach areas.[22] A recent systematic review concluded that powered toothbrushes are superior to manual toothbrushes in terms of removing plaque and improving gingival health but it reported a high level of heterogeneity among the studies.[23] One aspect to be addressed is the influence of individualized oral hygiene instructions on the comparisons of different toothbrushing systems. It is known that the ability to achieve low plaque levels by oral hygiene efforts depends on oral hygiene motivation and frequency.[24,25]

Therefore, the aim of this study was to compare the effectiveness of an intensive plaque control program with sonic or manual supervised toothbrushing in improving clinical and patient-centred outcomes, and in reducing GCF levels of MMP-1 and MMP-9 in patients affected by DG due to OLP.

Material and Methods

Experimental design and study population

The study was a parallel group, examiner-blinded, randomized, and controlled study conducted between March 2017 and July 2018. It was approved by the Institutional Ethics Committee of the "AOU Città della Salute e della Scienza", Turin, Italy (No. 0058273) and was conducted according to the Helsinki Declaration of 1975 (revised in 2000). The CONSORT guidelines for clinical trials were followed.

Caucasian patients attending the Oral Medicine Section at the University of Turin, C.I.R. Dental School, Department of Surgical Sciences were consecutively screened for enrolment according to the following criteria: adult patients aged 18 years or above; clinical and histological diagnosis of OLP on the basis of WHO criteria (hyperkeratosis of the

superficial epithelial layers, vacuolar degeneration of the germinative layer of the epithelium and band-like sub-epithelial lymphocytic inflammatory infiltrate)[26]; signs of DG; no treatment for OLP in the last 2 weeks in the case of topical treatments, or in the last 4 weeks in the case of systemic therapies.

Exclusion criteria were as follows: use of non-steroid anti-inflammatory medications and/or antibiotics in 8 weeks prior to the study; use of medications that could induce lichenoid reactions; smoking habits; patients suffering from severe destructive periodontal disease with at least one site with probing depth (PD) > 5 mm; patients unable to provide consent; pregnant or lactating women.

Sample size and randomization

After the baseline examination, the enrolled subjects were randomly assigned to two treatment protocols by using a computer-generated table. Patients in the test group were instructed for home-use of a sonic powered toothbrush (Philips FlexCare Platinum, Philips Oral Healthcare Inc., USA), while patients in the control group of a soft manual toothbrush (GUM Technique PRO Soft 525, Sunstar America Inc., USA). Allocation concealment was ensured by having the randomization performed by a person not involved in the study and by providing the dental hygienist with sealed envelopes containing assignment for individual patients. Investigators were blinded to the group assignment. The primary outcome of the study was change in the percentage of sites with bacterial plaque 8 weeks after starting the program. The sample size calculation led to 32 individuals (16 per arm), based on an expected difference between the two groups in plaque scores of 20% for an alpha of 0.05 and a power of 80%.

Clinical protocol

The study was articulated in 5 phases, for a total period of 8 weeks since patient recruitment. On the first visit (baseline, T0) detailed medical history and clinical data were collected by two calibrated and blinded clinicians (P.G.A. and F.F.). On the following day, before receiving delicate supragingival scaling and polishing by an experienced dental hygienist (L.Bo.) to avoid injuries to the gingival tissue, GCF samples were collected for

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biomolecular analysis. Patients were also asked to complete a pain-related questionnaire on a 10-cm horizontal visual analogue scale (VAS) and a questionnaire on the impact of the OLP on the quality of life. For this purpose, the Italian version of the OHIP-14 (Oral Health Impact profile) was used.[27]

After 1 week (T1), patients were clinically assessed again and they received by a separate hygienist (E.C.) careful instructions in self-performed plaque control measures: twice-daily toothbrushing using the sonic (manufacturer's usage instructions) or manual toothbrush (modified Bass technique) and once-daily interdental cleaning with extra-soft interdental devices according to the individual needs. The brushing time was set for 3 min for both the powered and manual toothbrushes as controlled by the use of a timer. The advice was to brush for 30 s the buccal and lingual/palatal surfaces of all teeth in a quadrant and thereafter to brush again for 60 s the buccal/lingual aspects of the DG affected sites. The specific verbal instructions for each type of toothbrush were followed by demonstration in the patient's mouth. Patients were invited to replicate demonstrated movements in their mouth under the supervision of the hygienist. The allocated toothbrush and fluoride-containing toothpaste without sodium lauryl sulphate (BioRepair Plus, Coswell Farma, Italy) were provided for use through the study.

A structured motivational plaque control was performed in the week 2, 3, and 4 after therapy (T2, T3, T4) to establish the most appropriate non-traumatic procedures and to obtain the best possible standard of oral hygiene. At these time points professional plaque removal was also performed. At 8-week follow up (T5) all clinical and biomolecular data were collected by the same examiner (P.A., F.R., N.G.). The VAS, the OHIP-14 and a questionnaire on the comfort were applied.

Clinical measurements

Full-mouth clinical periodontal measurements were recorded on six sites per tooth, using a 1-mm marked periodontal probe (PCP UNC15, Hu-Friedy, Chicago, IL, USA) and rounded to the nearest mm. The following periodontal parameters were recorded: presence/absence of bacterial plaque (O'Leary Plaque Score)[28]; bleeding index (BoP),

scored as positive if bleeding occurs after 15 s following probing, conversely negative, as reported by Guiglia et al.[18]; angulated bleeding score (AngBS)[29]; PD; gingival recession (REC); clinical attachment level (CAL).

Desquamative Gingivitis Clinical Score (DGCS), including the extent and severity of the gingival lesions, was also recorded at T1 and T5.[30]

GCF sampling and biomolecular analysis

For each patient GCF samples were collected using absorbent paper strips (Periopaper; Oraflow Inc., Smithtown, NY, USA) from one site with DG, PD ≤ 3 mm and clinical signs of inflammation (presence of plaque and BoP), and from one healthy control site (PD ≤ 3 mm, no BoP and no clinical signs of DG). The same sites were sampled at T0 and T5. Sites to be sampled were isolated with cotton rolls and air-dried. The paper strips were inserted 1mm into the crevice and left in place for 30 s. The volume of GCF was measured using a calibrated instrument (Periotron 8000, OraflowInc., Plainview, NY, USA) and converted in μl using MLCONVRT program.[31] Samples were subsequently stored at -80C until further enzyme processing was performed.

GCF samples were analysed for MMP-1 and MMP-9 using commercially available enzyme-linked immunosorbent assays (ELISAs; R&D System Inc., Minneapolis, MN, USA). Analyses were performed according to the manufacturer's protocol by a blinded examiner (M.M.). All ELISA determinations were performed in duplicate. Results were calculated using the standard curves created in each assay. The total level of cytokines in GCF was determined (pg/ml).

Statistical analysis

Data were first examined for normality by the Kolmogorov-Smirnov test, and if the data did not achieve normality, analyses were performed using non-parametric methods. The Wilcoxon test or the paired t-test was employed to detect statistically significant clinical and biomarkers differences within test and control group before and after the plaque control program. The Wilcoxon test was also used to evaluate changes over time in the patient-centred outcomes.

Differences between groups were tested using the unpaired t test or Mann-Whitney U-test for quantitative variables and Chi-square test for qualitative variables. The comparisons of MMP levels between healthy and diseased sites in test and control groups before and after the plaque control program were performed using the Kruskal-Wallis test, followed by post-hoc Dunn test. Statistical analyses were conducted with the significance level set at $P < 0.05$ using commercially available software (SPSS for Mac, SPSS version 24.0, IBM Corporation, Armonk, NY, USA).

Results

As reported in Figure 1, 49 OLP patients were screened for inclusion: 11 were excluded because they did not meet the inclusion criteria and 6 refused to participate. Finally, a total of 32 patients were enrolled in the study and randomly assigned to the sonic (n=16; mean age: 61.0 ± 9.3 years) or manual toothbrushing group (n=16; mean age: 65.4 ± 11.1 years). The oral manifestations of OLP were limited to the marginal and attached gingiva without other mucosal lesions. All patients completed all phases of this study and were included in the statistical analysis.

Clinical findings

The demographic and clinical data recorded for both groups at baseline and after 8 weeks are summarized in Tables 1 and 2. At baseline, the groups showed similarities with respect to age, gender distribution and clinical parameters ($P < 0.05$). No adverse effects were observed in any of the experimental groups.

The intensive plaque control program promoted a significantly reduction in mean values of plaque and bleeding scores compared to baseline in DG affected sites, regardless of which toothbrush system was used ($P < 0.001$). In both groups a general improvement in the number and severity of gingival lesions was confirmed by a statistically significant reduction in the DGCS values ($P < 0.005$). Intergroup comparison revealed at 8 weeks a lower percentage of PI- and BOP-positive sites as well as a higher index of improvement in the extent and severity of DG lesions in the sonic compared with the manual brush

group ($P \leq 0.007$).

Patient-centred outcomes

Table 3 presents the questionnaire data where self-perception was measured. The groups had similar baseline mean OHIP sum scores and pain-related VAS scores. At week 8 the distributions shift in both groups showed a statistically significant improvement in oral health quality of life and pain perception ($P \leq 0.002$). Although the sonic brush group experienced a greater overall improvement, the differences with the manual brush group did not reach statistical significance. The participants felt the sonic toothbrush was cleaning their teeth better and judged brushing more comfortable than the participants using the soft-bristle manual toothbrush did (15.7 ± 3.2 *versus* 12.4 ± 2.3, $P < 0.05$).

Biochemical activity

There was no statistically significant difference between the groups with respect to the enzymatic activity at baseline in both healthy and DG sites, as shown in Table 4. At the end of the oral hygiene protocol the GCF activity of MMP-1 and MMP-9 in diseased sites was no longer statistically different from healthy control sites in both groups ($P < 0.05$). The sonic brush group showed a statistically greater reduction in both MMP-1 ($P = 0.009$) and MMP-9 ($P = 0.027$) compared to the manual brush group.

Discussion

Although painful gingival and oral lesions can hinder proper oral hygiene practices and increase the possibility of dental plaque accumulation, there is a scarce literature about home oral care in OLP patients to achieve adequate plaque control and to improve severity of DG. To the best of our knowledge, this is the first study to evaluate concomitantly the effect of an intensive plaque control program with sonic or manual supervised toothbrushing on clinical outcomes and GCF levels of biomarkers associated with OLP. While a significant decrease in clinical indices, mucosal disease scores and biochemical activity was evident 8 weeks after an intensive individual hygiene treatment, the use of a sonic toothbrush achieved better results than the soft manual toothbrush did.

Previous clinical studies reported successful results in the management of gingival manifestations of OLP using different plaque control regimens. Holmstrup and coworkers instituted atraumatic oral hygiene procedures with a manual toothbrush combined with 0.12% chlorhexidine rinses, differently from the present study, in which the use of antiseptic mouthwashes was established as exclusion criteria.[17] In addition, patients were recalled on a three-month basis for 12 months. Similarly, Guiglia and coworkers combined 0.12% chlorhexidine mouthwashes for 7 days with topical corticosteroid therapy in a protocol that included instructions in the use of a soft-bristle toothbrush, supra and sub-gingival scaling.[18] Differently from the present study, patients were recalled monthly for 3 months only for reinforcement in oral hygiene procedures. Two more recent studies evaluated the effect of supervised plaque removal measures with soft manual toothbrush, in addition to supragingival scaling and tooth polishing with or without the administration of topical corticosteroids, over a 4 or 8-week period.[19,20] In spite of the differentiated methodologies, we observed an improvement in periodontal indices, painful symptoms, as well as extension and degree of the gingival lesions in line with that reported in the aforementioned studies, but similarly to others patients did not require any additional corticosteroid medication.[17,20]

As previously reported,[21] the sonic toothbrushing was more effective than manual toothbrushing in improving oral and periodontal status in DG patients. However, differently from the present study, the control group did not receive any additional intervention or advice and continued with their normal plaque control regimen.[21] Past investigations have shown the anti-plaque and anti-inflammatory benefits of sonic powered toothbrushes relative to manual toothbrushes for managing gingivitis and periodontitis.[32,33] Brushing-induced turbulence has been shown to drive fluid dynamic forces at a distance of 3 mm between the dental surface resulting in an effective biofilm removal into the more inaccessible areas of the oral cavity and causing minor gingival abrasions than a manual toothbrush.[22,34] This may be more relevant when managing painful atrophic mucosa that may discourage patients from brushing effectively.[2,35] Although it has been suggested that

plaque removal would potentiate new lesions resulting from mechanical trauma, however, this hypothesis lacks evidence.[7]

The structured plaque control intervention was also effective in improving oral health quality of life and pain perception. The 14-item version of the OHIP was used, showing to be reliable and responsive to the clinical changes.[36] The sonic group experienced a greater overall improvement in OHIP scores, and VAS scores for pain, nevertheless the differences with the manual toothbrushing group did not reach statistical significance. However, the participants felt the sonic toothbrush was cleaning their teeth better and judged brushing more comfortable than those using the soft toothbrush did. No adverse effect was observed. While bearing in mind the differences in the experimental design, it was also observed that the shift in the OHIP scores was more for the sonic than the control group, with the largest difference in the functional limitation, psychological discomfort and physical disabilities domains.[21] The cost-effectiveness of such structured plaque control intervention was previously reported.[37]

Interestingly, in the treatment of gingivitis and periodontitis, less invasive supragingival procedures had a greater impact on oral health-related quality of life then subgingival procedures.[38]

These promising clinical findings were also supported by biochemical analysis. Since it is known that the total amount of biomarkers varies based on the volume of GCF, the concentration instead of the total amount was considered.[39] For the present study, MMP-1 and MMP-9 were chosen as two inflammatory mediators actively involved in the connective tissue remodeling process.[10,11] Overproduction of MMPs has been demonstrated in inflamed gingival tissue from systemically healthy chronic periodontitis patients.[40,41] MMP-9 and MMP-1 have been also involved in the pathogenesis of OLP and their over-expression has been related to the degradation of the basal membrane and to the apoptosis of the overlying epithelium.[12,13] Higher levels of MMP-1 and MMP-9 and lower levels of their inhibitor TIMP-1 were observed in both the GCF and gingival tissue of OLP patients with gingivitis and periodontitis compared to non-OLP gingivitis and

periodontitis patients, in spite of comparable clinical parameters between groups.[9] This suggests that OLP may affect the periodontal status by changing the MMPs levels and thus more breakdown could be occurring in the long-term course of the disease. Interestingly, the structured plaque control intervention was effective in decreasing the GCF activity of MMP-1 and MMP-9 in DG sites to a level not statistically different form that of healthy control sites. The sonic group showed a statistically greater reduction in both MMPs compared to the manual group. It is reasonable to hypothesize that the efficacy of daily oral hygiene procedures prevents periodontal breakdown and reduces the local inflammatory reaction and the activation of the immune system stimulated by bacterial factors.[42]

Despite the objective and subjective benefits of the model, it may be influenced by the 'Hawthorne effect'. This phenomenon may have modified the behavior of the study participants purely as a result of them knowing that they are enrolled into a clinical trial.[43] In addition, the duration of follow-up was limited to 8 weeks. Therefore, the obtained results should be interpreted with caution and should be extrapolated only to population with DG and high motivation. Patients were highly motivated to properly perform the oral hygiene procedures at home, and their cooperation was essential. It is known that oral status impacts how individuals perceive their quality of life.[44] Lastly the duration of toothbrushing is an important determinant of plaque removal.[45-47] Since sonic toothbrushes have built-in timers, their use may have facilitated patients in controlling the brushing time. This may partly explain the higher effect of powered toothbrushing on the plaque score reduction.

Conclusion

This clinical trial reported evidence of the effectiveness of a combined protocol based on professional oral hygiene and supervised toothbrushing procedures in improving clinically observed gingival manifestations of DG and oral health-related quality of life in OLP patients. This could be attributable to the time dedicated by the operator to oral hygiene performance and home instructions. Dental hygienists should deliver intensive plaque

control programs during the initial phase of DG assetment, and check on a regular basis the patient adherence to a proper daily oral hygiene regimen. Within the limitations of the present study, the daily use of a sonic toothbrush would seem to perform better than a manual soft-bristle toothbrush in the short term. In any case, manual toothbrushes still represent an option expecially for patients with low socioeconomic status. Long-term studies are needed to confirm the results obtained.

Clinical relevance

Scientific rationale for study: There is a lack of investigations evaluating the clinical and biochemical impact of intensive plaque control programs in patients with desquamative gingivitis (DG) due to oral lichen planus (OLP).

Principal findings: Sonic toothbrushing was more effective than manual soft-bristle toothbrushing in improving oral and periodontal status in DG patients in the short term.

Practical implications: Sonic-powered toothbrushing may be a useful device for plaque control in OLP patients with DG provided that a tailored supervised toothbrushing program is instituted.

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Table 1. Mean (± SD) demographic and clinical parameters in DG affected sites at baseline for the two treatment groups.

	Brushing group		*P*-value sonic vs manual
	Sonic brush (*n* = 16)	Manual brush (*n* = 16)	
Age (years)	61.0 ± 9.3	65.4 ± 11.1	0.897
Female/male	9/7	11/5	0.465
Sites with plaque (%)	88.7 ± 12.5	79.7 ± 19.3	0.130
Sites with BOP (%)	63.4 ± 27.7	56.7 ± 24.5	0.475
AngBS	0.6 ± 0.5	0.5 ± 0.3	0.435
PD (mm)	2.9 ± 0.5	3.1 ± 0.4	0.331
DGCS	8.8 ± 2.7	9.2 ± 2.8	0.708

BOP presence of bleeding on probing, *AngBS* angulated bleeding score, *PD* probing depth, *DGCS* desquamative gingivitis clinical score, *SD* standard deviation.

Table 2. Changes in clinical parameters in DG affected sites (mean ± SD) over the 8-week experimental period.

Variable	Brushing Group	Baseline (T0)	8 weeks (T5)	Δ_{T0-T5}	P-value T0-T5[a]
Sites with plaque (%)	Sonic brush	88.7 ± 12.5	26.5 ± 11.7	62.2 ± 16.1	<0.001
	Manual brush	79.7 ± 19.3	47.2 ± 15.8	32.5 ± 17.9	<0.001
P-value sonic vs manual[b]		NS	<0.001		
Sites with BoP (%)	Sonic brush	63.4 ± 27.7	20.6 ± 11.2	42.8 ± 22.5	<0.001
	Manual brush	56.7 ± 24.5	33.4 ± 9.2	23.3 ± 20.4	<0.001
P-value sonic vs manual[b]		NS	0.001		
AngBS	Sonic brush	0.6 ± 0.5	0.2 ± 0.1	0.4 ± 0.5	<0.005
	Manual brush	0.5 ± 0.3	0.3 ± 0.1	3.6 ± 0.8	<0.01
P value sonic vs manual[b]		NS	0.014		
PD (mm)	Sonic brush	2.9 ± 0.5	2.7 ± 0.6	0.2 ± 0.4	
	Manual brush	3.1 ± 0.4	2.8 ± 0.3	0.2 ± 0.3	
P-value sonic vs manual[b]		NS	NS		
DGCS	Sonic brush	8.8 ± 2.8	4.0 ± 1.7	4.8 ± 2.7	<0.001
	Manual brush	9.2 ± 2.8	6.1 ± 2.3	3.1 ± 2.9	<0.001
P-value sonic vs manual[b]		NS	0.007		

BOP presence of bleeding on probing, *AngBS* angulated bleeding score, *PD* probing depth, *DGCS* desquamative gingivitis clinical score.
NS = difference between groups is not statistically significant (*P* > 0.05)
[a]Paired t-test or Wilcoxon test
[b]Mann-Withney *U*-test or unpaired *t*-test

Table 3. Patient-centered outcomes for the two treatment groups.

	Baseline (T0)		8 weeks (T5)		
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	*P*-value T0-T5[a]
PAIN (VAS score)					
Sonic brush	5.4 ± 2.3	5.5 (9.0)	2.3 ± 2.4	1.5 (4.75)	<0.001
Manual brush	5.3 ± 2.4	5.0 (7.0)	2.9 ± 2.3	2.5 (4.5)	<0.001
P-value sonic vs manual[b]	0.824		0.739		
OHIP-14					
Sonic brush	13.9 ± 8.8	13.5 (15.5)	7.0 ± 5.5	6.0 (8.0)	0.002
Manual brush	15.6 ± 9.2	15.0 (15.5)	8.6 ± 4.8	8.5 (6.25)	0.001
P-value sonic vs manual[b]	0.939		0.858		

[a]Wilcoxon test
[b]Mann-Withney *U*-test

Table 4. Changes in biochemical parameters (mean ± SD) over the 8-week experimental period in GCF sampling sites.

	Brushing group					
	Sonic brush (*n* = 16)			Manual brush (*n* = 16)		
	Healthy sites	DG sites		Healthy sites	DG sites	
		Baseline	8 weeks		Baseline	8 weeks
MMP-1 (pg/ml)	92.04 ±32.84	219.20 ± 60.21[a]	92.29 ±16.27[b]	109.10 ± 40.61	243.04 ±134.98[a]	122.41±38.71[b]
MMP-9 (pg/ml)	5.07 ± 2.87	10.21 ± 2.87[a]	4.81 ± 1.67[b]	6.38 ± 5.10	10.65 ± 4.26[a]	6.88 ± 3.17[b]
GCF volume (µl)	0.22 ± 0.15	0.64 ± 0.36[a]	0.27 ± 0.17[b]	0.28 ± 0.11	0.59 ± 0.33[a]	0.37 ± 0.20[b]

MMP-1 matrix metalloproteinase-1, *MMP-9* matrix metalloproteinase-9, *GCF* gingival crevicular fluid, *SD* standard deviation.
[a] Significantly different from healthy sites, $P < 0.001$.
[b] Significantly different from baseline, $P < 0.001$.

Fig.1 Consort diagram showing the study design


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                    ┌─────────────────────────┐
                    │   Screened (n=49)       │
                    └─────────────────────────┘
                              │
                              │         ┌──────────────────────────────────────────┐
                              │────────▶│ Excluded  (n=17)                          │
                              │         │  ♦ Not meeting inclusion criteria (n= 11) │
                              │         │  ♦ Declined to participate (n=6)          │
                              │         │  ♦ Other reasons (n= 0)                   │
                              │         └──────────────────────────────────────────┘
                              ▼
                    ┌─────────────────────────┐         ┌──────────────────┐
                    │   Eligible (n=32)       │────────▶│ Excluded  (n=0)  │
                    └─────────────────────────┘         └──────────────────┘
                              │
                              ▼
                    ┌─────────────────────────┐
                    │   Randomized (n=32)     │
                    └─────────────────────────┘
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Allocated to intervention sonic toothbrushin (n= 16)	Allocated to intervention manual toothbrush (n=16)
♦ Received allocated intervention (n=16)	♦ Received allocated intervention (n=16)
♦ Did not receive allocated intervention (n=0)	♦ Did not receive allocated intervention (n= 0)

8-week Follow-Up

Lost to follow-up (n=0)	Lost to follow-up (n= 0)

Analysis

Analysed (n= 16)	Analysed (n= 16)
♦ Excluded from analysis (n= 0)	♦ Excluded from analysis (n= 0)