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Short-term microbiological effects of scaling and root planing and essential-oils mouthwash in Chinese adults*

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Abstract: Objective: To assess the short-term effect of scaling and root planing (SRP) and essential-oils mouthwash on the levels of specific bacteria in Chinese adults. Methods: Fifty Chinese adults with chronic periodontitis were randomly assigned to full-mouth SRP or a 7-d essential-oils mouthwash regimen. In addition, 22 periodontally healthy adults used essential-oils mouthwash for 7 d. Clinical examination and plaque/saliva sampling were performed at baseline and on Day 7. Quantitative real-time polymerase chain reaction (PCR) was used to measure *Aggregatibacter actinomycetemcomitans* (*Aa*), *Fusobacterium nucleatum* (*Fn*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), and total bacterial loads in saliva, supra- and sub-gingival plaque samples. Results: The detection frequencies of four tested species remained unchanged after either treatment. However, the bacterial loads of *Fn, Pg*, and *Pi* were significantly reduced by SRP; the mean reduction of bacterial counts in saliva ranged from 52.2% to 62.5% ($p<0.01$), in supragingival plaque from 68.2% to 81.0% ($p<0.05$), and in subgingival plaque from 67.9% to 93.0% ($p<0.01$). Total bacterial loads were reduced after SRP in supra- and sub-gingival plaque ($p<0.05$). Essential-oils mouthwash reduced *Fn* levels in supragingival plaque by a mean of 53.2%, and reduced total bacterial loads in supra- and sub-gingival plaque ($p<0.01$). In subgingival plaque from periodontal patients, *Pg* and *Pi* reductions were high after SRP compared to essential-oils mouthwash (93.0% vs. 37.7% and 87.0% vs. 21.0%, $p<0.05$). No significant bacterial reduction was observed in periodontally healthy subjects using essential-oils mouthwash. Conclusions: SRP and essential-oils mouthwash both have an impact on saliva and gingival plaque flora in Chinese periodontitis patients in 7 d, with greater microbiological improvement by SRP.

Key words: Oral microbiota, Chronic periodontitis, Scaling and root planing, Essential-oils, Quantitative detection, Real-time PCR

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1 Introduction

Chronic periodontitis (CP), the most common periodontal disease, causes an inflammatory host response leading to bone loss and connective tissue destruction. The initiation and progression of CP are the result of oral bacteria and the host immune response. Approximately 700 different bacterial species can inhabit the human oral cavity (Paster *et al.*, 2006). Of these species, *Aggregatibacter actinomycetemcomitans* (*Aa*), *Fusobacterium nucleatum* (*Fn*), *Porphyromonas gingivalis* (*Pg*), and *Prevotella intermedia* (*Pi*) are closely associated with periodontal diseases (Armitage, 2010). Periodontal therapy, which is

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aimed at controlling inflammatory processes and improving clinical parameters, has been mainly used to alter the periodontal environment by reducing or eliminating periodontopathogens in the dental plaque (Haffajee *et al.*, 2006).

Anti-infective nonsurgical therapy includes both mechanical and chemotherapeutic approaches. Regular chemotherapeutic home-care by patients, such as applying chlorhexidine and essential-oils mouthwash, is generally very effective in controlling most inflammatory periodontal diseases, when used in conjunction with the professional removal of dental plaque (Drisko, 2001). Chlorhexidine is a well-known antiseptic for antiplaque action with a long substantivity, but side-effects such as extensive tooth staining, taste alteration, and calculus deposition limit patient compliance for long-term use. Essential-oils mouthwash has been shown to help in reducing periodontopathogens and in controlling plaque and gingivitis (Fine *et al.*, 1994; Pizzo *et al.*, 2008) and may be a reliable alternative to chlorhexidine regarding gingival inflammation for long-term use (van Leeuwen *et al*., 2011). The use of an essential-oils mouthwash has been recommended as adjunctive therapy in orthodontic patients with less than optimal oral hygiene, in medically compromised patients, and in patients at high risk of periodontal diseases (Barnett, 2006). However, for CP patients, the clinical benefits of mouthwash alone are limited, and mechanical treatment is effective in reducing the bacterial loads resulting in clinical improvement (Slots *et al.*, 1979; Umeda *et al.*, 2004). Scaling and root planing (SRP) is the first-mode mechanical treatment recommended for most periodontal infections and remains an essential part of successful periodontal therapy (Cobb, 2002). SRP alone or with antiseptic yields clinical benefits (Haffajee *et al.*, 1997; Heitz-Mayfield *et al.*, 2002; Faveri *et al.*, 2006).

Although beneficial effects of SRP and essential-oils mouthwash on plaque control and clinical assessment have been reported, few studies have assessed their microbiological effect before and after treatment. Microbiological monitoring or evaluation is a useful supplement to clinical measurement to guide treatment strategies and achieve good treatment outcomes. After non-surgical periodontal treatment, oral niches in favor of periodontal bacteria can shift from a predominantly Gram-negative to a Gram-positive subgingival microbiota. However, currently very little data are available in the literature on the microbiological outcome after non-surgical periodontal therapy in the Chinese population (Xu *et al.*, 2004; Cheng *et al.*, 2008). Moreover, while previous studies assessing the microbiological effects of periodontal therapy mainly used the techniques of culture, DNA probe or polymerase chain reaction (PCR), recent oral research has used quantitative real-time PCR (qRT-PCR), due to its accurate and rapid assessment of microbiological levels (Gomes *et al.*, 2008; Cionca *et al.*, 2010).

The aim of this study was to quantify and evaluate the short-term microbiological effects following either SRP or essential-oils mouthwash in Chinese adults with CP. Furthermore, we aimed to examine the microbiological effect of essential-oils mouthwash in both periodontally healthy and diseased Chinese adults. We applied qRT-PCR based on 16S rRNA genes to assess the detection frequencies and bacterial loads of *Aa*, *Fn*, *Pg*, *Pi*, and total bacteria in saliva, supra- and sub-gingival plaque at baseline and after 7 d.

2 Materials and methods

2.1 Participants and clinical assessment

Fifty CP patients, between the ages of 19 and 58 years who visited the Affiliated Hospital of Stomatology at Zhejiang University, were enrolled in this study on a rolling basis within a window of six months. An additional 22 periodontally healthy volunteers 20 to 46 years of age were recruited. Clinical measurements such as probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) were made at six sites per tooth (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual, and lingual-distal). CP patients exhibited BOP, with at least one or more sites of CAL $\geq$4 mm, four sites of PD $\geq$4 mm, and radiographic alveolar bone loss in four or more teeth. Periodontally healthy subjects showed no clinical evidence of periodontal disease. Subjects were excluded if they (1) had used mouthwash in the previous year, (2) had used antibiotics within the last three months, (3) had received periodontal treatment in the previous six months, (4) were pregnant or had medical conditions such as heart

disease and hypertension that may influence the progression of periodontal disease, or (5) had less than 20 teeth.

This study was reviewed and approved by the Ethics Board of the Affiliated Hospital of Stomatology in Zhejiang University. All participants were informed about the study design and signed informed consent forms.

2.2 Sample collection

Saliva, supra- and sub-gingival plaque samples were collected from each subject at the start of the treatment session. Subjects refrained from eating, drinking, and brushing for at least one hour prior to sample collection. Each subject was instructed to expectorate into an aseptic paper cup, and 500 μl saliva was transferred into a sterile 1.5 ml microcentrifuge tube. Supra- and sub-gingival plaque samples were collected from four teeth using a standardized method (Herrera *et al.*, 2008). The teeth to be sampled were isolated with sterile cotton rolls and gentle air drying. Supragingival plaque samples were collected from the mesial-buccal surface of the first molar (the second molar if the first molar was missing) in each quadrant. No participant in this study had both the first and second molars missing. Supragingival plaque samples were carefully scraped with sterile explorers from the gingival margin to prevent contamination with subgingival plaque, and the pooled supragingival sample from each subject was placed in a microcentrifuge tube containing 1.5 ml sterile reduced transfer fluid (RTF) buffer following Syed and Loesche (1972). Subgingival plaque was sampled from the site of the deepest pocket of the tooth from which supragingival plaque was collected. A #30 sterile paper point was gently inserted into the periodontal pocket for 20 s and immediately removed to RTF buffer. Four paper points from each subject were pooled in the same microcentrifuge tube with RTF buffer. All samples were stored at −80 °C immediately after collection.

2.3 Treatments

After verifying their eligibility and sampling their saliva and plaque, CP patients were randomly assigned to receive SRP (N=25) or essential-oils mouthwash (Listerine, Johnson & Johnson, Bangkok, Thailand) (N=25). Random allocation was carried out using a simple coin-tossing method. Periodontally healthy subjects (N=22) received mouthwash treatment. For subjects assigned to receive SRP, single-visit one-stage full mouth dental debridement was performed using periodontal ultrasonic scaling (Satelec P5, Mérignac, France) without anesthesia. Subjects receiving essential-oils mouthwash were instructed to rinse with 20 ml mouthwash for 30 s twice a day for 7 d. All subjects maintained their own routine oral hygiene procedures during the 7-d study-period.

BOP measurement was recorded for all patients returning to the hospital on the 7th day after treatment. In addition, saliva, supra- and sub-gingival plaque samples were collected using the same procedure as the initial visit.

2.4 Microbiological examination: qRT-PCR

A total of 432 samples (72 each of saliva, supra- and sub-gingival plaque before and after treatment) were collected and qRT-PCR was used to assess the content of the four targeted species, *Aa*, *Fn*, *Pg*, *Pi*, and total bacteria as described by He *et al.* (2012). Briefly, DNA from the samples and cultivated *Aa* ATCC 29523, *Fn* ATCC 25586, *Pg* ATCC 33277, and *Pi* ATCC 25611 were isolated using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA concentration of cultivated bacterial strains was quantified by a Nanodrop 1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, USA) and the number of bacterial cell copies was quantified and calculated based on the molecular mass formula according to the method of Novak *et al.* (2008). Quantification of target species was achieved by using serial ten-fold dilutions from 10^2 to 10^7 cell copies of the quantified standard for each bacterium in the qRT-PCR analysis. Bacteria-specific primer pairs and a universal primer pair according to the literature based on the 16S rRNA gene were used to quantify each target bacterium and the total bacteria in samples (Baumgartner *et al.*, 1999; Sakamoto *et al.*, 2001; Nadkarni *et al.*, 2002; Maeda *et al.*, 2003; Suzuki *et al.*, 2004). All samples were run in duplicate in 96-well plates in a LightCycler® 480 Real-Time PCR system (Roche Diagnostics, Mannheim, Germany). Samples were assayed in a 10 μl reaction mixture containing 1 μl template DNA, 5 μl LightCycler® 480 SYBR Green I Master, 1 μl 2.5 μmol/L primer pair, and 3.5 μl ddH_2O. The amplification cycling conditions

were 95 °C for 10 min, 40 cycles of 10 s at 95 °C, 15 s at a bacterium-specific annealing temperature, and 40 s at 72 °C. Melting analysis was performed for each run to verify the specificity of the PCR products.

2.5 Statistical analysis

The demographic and clinical characteristics of the two CP groups were compared using standard univariate statistics (*t*-test and Fisher's exact test). The BOP was assessed for the change from baseline to 7 d using the paired *t*-test.

The main microbiological outcomes of interest were changes in detection frequencies and bacterial loads in saliva, supra- and sub-gingival plaque after 7 d of treatment. The absolute bacterial counts were $\log_{10}$ transformed for analysis, and 1 was added to all the bacteria counts to circumvent issues of zero values with the $\log_{10}$ transformation. Microbiological effectiveness of treatment was analyzed by applying the paired *t*-test to the detection frequencies and $\log_{10}$ transformed bacterial counts before and after treatment. Furthermore, the percentage reduction was calculated by first subtracting the bacterial counts 7 d after treatment from the counts at baseline and then dividing by those at baseline. Wilcoxon rank-sum test was used to compare bacterial percentage reductions between SRP and essential-oils mouthwash in CP patients. Quantile regression was used to evaluate bacterial percentage reductions between the CP and periodontally healthy subjects receiving essential-oils mouthwash, adjusting for age and gender, as age and gender ratio were significantly different between the CP and periodontally healthy groups.

We determined the number of subjects in the treatment regimen based on historical data, and the sample size (≥21 participants in each group) was calculated to provide 90% power to detect one standard difference of $\log_{10}$ transformed bacterial counts between treatment groups. All tests were two-sided with a significance level of $p<0.05$. All analyses were conducted with SAS V. 9.2.

3 Results

The demographic and clinical characteristics of 72 participants at baseline are listed in Table 1. No significant differences in age, gender, smoking history, BOP, PD, or CAL were observed between the SRP and essential-oils mouthwash groups of CP patients ($p>0.12$). The BOP improved in both the CP treatment groups on Day 7. CP patients who received SRP showed a reduced mean percentage of sites with BOP from 34.7% to 23.5% ($p=0.001$) and those who received essential-oils mouthwash showed a reduction from 43.0% to 25.9% ($p<0.001$). None of the participants reported adverse experiences during the 7 d.

In the CP patients, neither treatment was followed by a significant reduction in the detection frequency ($p>0.08$, Table 2). *Fn* was detected in all the CP patients before and after treatment. Both *Pg* and *Pi* were still present, with detection frequencies of 92.0% (23/25) and 100% (25/25), respectively, in both the SRP and essential-oils mouthwash treatment groups. *Aa* was detected ≤50% at baseline and after treatment. Although they did not completely remove these four bacteria, to some extent both treatments reduced bacterial loads in CP patients (Fig. 1). The $\log_{10}$ transformed bacterial counts before and after treatment on Day 7 for four species and total bacteria are listed in Table 3. SRP reduced the *Fn*, *Pg*, and *Pi* in all samples of saliva, supra- and sub-gingival plaque;

Table 1 Baseline characteristics and clinical parameters of the study population

Group	Age (year)	n_{male}	n_{smoker}	Site with BOP (%)	PD (mm)	CAL (mm)	PD_s (mm)	CAL_s (mm)
Chronic periodontitis patients								
SRP (*N*=25)	31.0±6.8	21 (84%)	7 (28%)	34.7±16.3	2.5±0.6	1.8±1.4	3.8±0.6	3.6±1.4
Mouthwash (*N*=25)	34.3±10.1	19 (76%)	10 (40%)	43.0±18.8	2.5±0.5	2.2±1.4	3.9±0.7	3.9±1.6
Healthy subjects								
Mouthwash (*N*=22)	26.2±6.1*	10 (45.5%)*	3 (13.6%)	13.3±15.0*	1.6±0.4*	0.1±0.1*	2.4±0.4*	0.0±0.1*

Data are expressed as mean±standard deviation (SD) or number (percent). n_{male}: number of males; n_{smoker}: number of smokers; BOP: bleeding on probing; PD: probing depth; CAL: clinical attachment level. Mean PD (CAL) of a subject was calculated by averaging the PD (CAL) of all measured sites, with six sites per tooth, in the full mouth. PD_s: PD of four sampling sites; CAL_s: CAL of four sampling sites. * $p<0.05$ between the CP and periodontally healthy groups

Table 2 Detection frequencies of four species of periodontal bacteria

Organism	Chronic periodontitis patients				Healthy subjects	
	SRP (*N*=25)		Mouthwash (*N*=25)		Mouthwash (*N*=22)	
	Baseline	7 d	Baseline	7 d	Baseline	7 d
Aa						
Saliva	9	7	14	12	7	5
Supragingival plaque	7	6	11	8	7	5
Subgingival plaque	3	2	8	6	2	2
Fn						
Saliva	25	25	25	25	22	22
Supragingival plaque	25	25	25	25	22	22
Subgingival plaque	25	25	25	25	22	22
Pg						
Saliva	23	23	23	23	10	10
Supragingival plaque	23	22	23	23	10	10
Subgingival plaque	23	22	23	23	9	7
Pi						
Saliva	25	25	25	25	18	16
Supragingival plaque	25	25	25	24	15	14
Subgingival plaque	25	24	24	24	13	9

Aa: *Aggregatibacter actinomycetemcomitans*; *Fn*: *Fusobacterium nucleatum*; *Pg*: *Porphyromonas gingivalis*; *Pi*: *Prevotella intermedia*; SRP: scaling and root planing

Table 3 Mean $\log_{10}$ transformed bacterial counts at baseline and on Day 7

Organism	Chronic periodontitis patients						Healthy subjects		
	SRP (*N*=25)			Mouthwash (*N*=25)			Mouthwash (*N*=22)		
	Mean (SE)		Reduction (%)	Mean (SE)		Reduction (%)	Mean (SE)		Reduction (%)
	Baseline	7 d		Baseline	7 d		Baseline	7 d	
Aa									
Saliva	1.5 (2.2)	1.2 (2.1)	46.5	2.5 (2.4)	2.1 (2.4)	57.1	1.4 (2.2)	1.0 (2.0)	58.6
Supragingival plaque	0.9 (1.6)	0.9 (1.8)	4.0	1.6 (2.0)	1.5 (2.3)	23.7	1.0 (1.7)	0.8 (1.6)	40.2
Subgingival plaque	0.5 (1.5)	0.4 (1.4)	24.4	1.4 (2.2)	1.0 (2.0)	55.2	0.4 (1.2)	0.5 (1.5)	−19.6
Fn									
Saliva	6.6 (0.3)	6.3 (0.5)	52.2	6.4 (0.5)	6.4(0.5)	17.3	6.1 (0.6)	6.1 (0.6)	8.2
Supragingival plaque	7.3 (0.3)	6.8 (0.6)	68.2	7.3 (0.3)	7.0 (0.4)	53.2	6.8 (0.9)	6.6 (0.7)	30.2
Subgingival plaque	6.3 (0.6)	5.8 (0.7)	67.9	6.3 (0.5)	6.2 (0.5)	26.8	5.2 (0.8)	5.2 (1.0)	11.9
Pg									
Saliva	6.6 (2.0)	6.1 (1.9)	62.5	6.5 (2.1)	6.3 (2.0)	38.1	2.8 (3.2)	2.8 (3.2)	2.6
Supragingival plaque	5.8 (1.9)	5.1 (2.2)	81.0	6.2 (2.1)	6.1 (2.0)	17.6	2.4 (2.8)	2.5 (2.9)	−37.0
Subgingival plaque	5.9 (2.0)	4.7 (2.1)	93.0	5.9 (2.2)	5.7 (2.1)	37.7	1.9 (2.5)	1.7 (2.6)	37.6
Pi									
Saliva	6.3 (0.5)	6.0 (0.6)	53.6	5.8 (0.7)	5.7 (0.7)	28.4	3.7 (2.1)	3.3 (2.2)	65.9
Supragingival plaque	6.1 (1.1)	5.5 (0.9)	72.1	5.9 (1.1)	5.6 (1.7)	52.9	2.9 (2.3)	3.0 (2.4)	−7.4
Subgingival plaque	5.0 (1.1)	4.1 (1.3)	87.0	4.5 (1.5)	4.4 (1.6)	21.0	1.9 (1.8)	1.3 (1.7)	70.8
Total									
Saliva	9.3 (0.2)	9.2 (0.3)	20.0	9.1 (0.5)	9.0 (0.6)	11.0	9.0 (0.5)	8.9 (0.5)	22.8
Supragingival plaque	9.1 (0.2)	8.9 (0.4)	33.8	9.1 (0.3)	8.8 (0.5)	57.2	8.8 (0.7)	8.7 (0.4)	20.7
Subgingival plaque	7.8 (0.4)	7.4 (0.6)	58.1	8.1 (0.4)	7.7 (0.4)	56.4	6.9 (0.6)	6.8 (0.9)	22.3

Aa: *Aggregatibacter actinomycetemcomitans*; *Fn*: *Fusobacterium nucleatum*; *Pg*: *Porphyromonas gingivalis*; *Pi*: *Prevotella intermedia*; SRP: scaling and root planing; SE: standard error

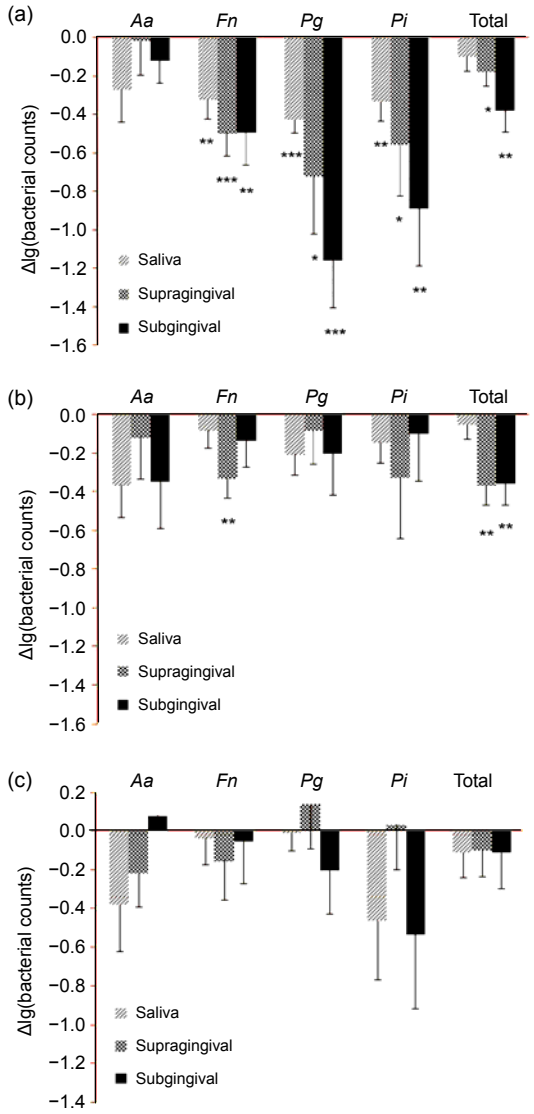

Fig. 1 Mean change in $\log_{10}$ transformed bacterial counts between baseline and 7 d post-treatment

(a) SRP in CP patients; (b) Essential-oils mouthwash in CP patients; (c) Essential-oils mouthwash in periodontally healthy subjects. * p<0.05, ** p<0.01, *** p<0.001 between baseline and 7 d post-treatment. Means with standard error bars are presented

the mean reductions in bacterial counts in saliva were 52.2%, 62.5%, and 53.6%, respectively (p<0.01); the mean reductions in supragingival plaque were 68.2%, 81.0%, and 72.1% (p<0.05); and in subgingival plaque 67.9%, 93.0%, and 87.0% (p<0.01). After SRP, the total bacterial loads were reduced in supragingival plaque (mean 33.8%, p=0.02) and subgingival plaque (mean 58.1%, p=0.003). Essential-oils mouthwash reduced the *Fn* levels in supragingival plaque (mean 53.2%, p=0.004), and total bacteria in supragingival plaque (mean 57.2%, p=0.001) and subgingival plaque (mean 56.4%, p=0.003). Moreover, the percentage reductions of *Pg* and *Pi* in subgingival plaque in the SRP group were greater than those in CP patients who received essential-oils mouthwash (93.0% vs. 37.7%, p=0.004, and 87.0% vs. 21.0%, p=0.049, respectively).

The periodontally healthy subjects who received essential-oils mouthwash showed no significant differences in the bacterial detection frequencies and bacterial loads during the 7-d follow-up period (Tables 2 and 3). Furthermore, there was no statistically significant difference in the percentage reduction between CP patients and periodontally healthy subjects receiving essential-oils mouthwash.

4 Discussion

In the present study, the presence of four target bacteria was not influenced by either SRP or essential-oils mouthwash in 7 d. However, the bacterial loads on Day 7 were greatly reduced in CP patients treated with SRP. Essential-oils mouthwash showed some changes in the bacterial loads of CP patients but no change in periodontally healthy participants.

That there was no difference in the presence of bacteria before and after SRP in this study is consistent with the report of Xu *et al.* (2004), who used PCR and reverse hybridization assay and observed no difference in the presence of *Aa*, *Pg*, *Pi*, *Tannerella forsythensis*, or *Treponema denticola* in 20 CP patients in Hong Kong treated with SRP and adjunct hyaluronic acid gel. Darby *et al.* (2001) also showed that the detection frequencies of these periodontal pathogens remained the same after SRP treatment using the PCR technique. In contrast, some groups reported significant reduction of the presence of periodontal pathogens after SRP using the checkerboard DNA-DNA hybridization or culture technique (Haffajee *et al.*, 1997; Bollen *et al.*, 1998; Colombo *et al.*, 2005). Discrepancies in the bacterial detection frequencies between different studies may be attributed to geographic differences, sampling procedures, or detection/enumeration methods. Teles *et al.* (2006) explicitly discussed the impact of sampling strategies and detection techniques on the microbiological

outcomes of previous studies of periodontal therapies and suspected that these factors largely account for the seeming discrepancies. In the paper cited above, Teles *et al.* (2006) also pointed out that the most frequent microbiological outcome of periodontal therapy appears to be a significant reduction in the levels of periodontopathogens, although the most desirable effect would be to eradicate those species. As they suggested, the microbiological goal of periodontal therapy is to lower the periodontal bacterial loads to an extent that is both compatible with the host and can be maintained by clinicians and periodontal patients. In our study, although no significant difference in detection frequencies was found, SRP significantly reduced the counts of the periodontal pathogens *Fn*, *Pg*, and *Pi*, but not *Aa*, which is consistent with the work of others (Doungudomdacha *et al.*, 2001; Pawlowski *et al.*, 2005). Moreover, the fact that no difference in the amount of *Aa* was found after SRP in our study is also consistent with a study by Fujise *et al.* (2002), which found that SRP did not significantly change the level of *Aa* using the qRT-PCR technique. The observed difference in bacterial loads but not presence after periodontal treatment in this study supports the idea that the levels of bacterial species, rather than their presence, are important in evaluating periodontal therapeutic effects (Haffajee *et al.*, 2006).

The anti-plaque and anti-gingivitis effectiveness of essential-oils mouthwash on dental plaque control has previously been demonstrated under different clinical conditions (Charles *et al.*, 2001; Seymour, 2003; Sharma *et al.*, 2004). Consistent with the work of others, we found that essential-oils mouthwash reduced the levels of total bacteria and *Fn* in 7 d, without significantly reducing the detection frequencies. The percentage reduction of *Fn* observed in supragingival plaque in CP patients is consistent with the findings of a previous randomized clinical trial investigating the effect of essential-oils mouthwash on the levels of plaque flora (Fine *et al.*, 2007).

It is widely accepted that various periodontal pathogens are associated with periodontal disease status (Socransky and Haffajee, 2005). We hypothesized that essential-oils mouthwash may have different effects on microbiological outcomes between CP patients and periodontally healthy subjects. Indeed, we found no significant change of bacterial loads in any of the four target bacterial species and total bacterial loads in periodontally healthy participants after a 7-d course of essential-oils mouthwash, while some changes in bacterial loads occurred in the CP patients after using this mouthwash. The stable bacterial loads of periodontally healthy subjects before and after using essential-oils mouthwash may be explained by the presence of a beneficial bacterial etiology in the healthy subjects, and the targeting on certain microorganisms in the oral cavity (Fine *et al.*, 2000; Stoeken *et al.*, 2007).

Periodontopathogenic bacteria can colonize multiple ecological sites with varied distributions (Mager *et al.*, 2003). Bacteria in supragingival plaque or saliva can have an impact on the subgingival re-colonization of pockets after periodontal therapy, and the intraoral translocation of periodontal pathogens may jeopardize periodontal therapy (Quirynen *et al.*, 1996; 2001). We assessed four target bacterial species in saliva, supra- and sub-gingival plaque before and after SRP and found that in all these locations *Fn*, *Pg*, and *Pi* were reduced, indicating that subgingival plaque control is important and affects the overall distribution of periodontal pathogens in the oral niches.

Essential-oils mouthwash was approved by the American Dental Association for supragingival plaque control and anti-gingivitis in 1987, and it has become available over-the-counter in China in recent years. Even though the clinical and microbiological benefits of essential-oils mouthwash alone or as an adjunct to periodontal therapies on Europeans and North Americans have been reported, to the best of our knowledge, no published study to date has evaluated the effect of essential-oils mouthwash in the Chinese population using qRT-PCR. In this study, essential-oils mouthwash was assigned to participants for using in conjunction with unsupervised daily oral hygiene in an attempt to simulate the normal daily situation. Besides treating a group of periodontally healthy subjects with essential-oils mouthwash, a group of CP patients was also treated with this mouthwash for the following reasons. First, essential-oils mouthwash alone has been shown to improve clinical outcomes such as inflammation and reduce periodontopathogens in patients with periodontitis (Fine *et al.*, 2007; Haffajee *et al.*, 2009). Second, using essential-oils mouthwash may be a good home-care practice for Chinese adults to improve their oral hygiene,

particularly for people whose oral hygiene is compromised because of temporary disability or lack of awareness. Third, this study was intended to compare the microbiological effect of essential-oils mouthwash between CP patients and periodontally healthy subjects.

In conclusion, SRP significantly reduced the bacterial loads of *Fn*, *Pg*, *Pi*, and total bacteria in saliva, supra- and sub-gingival plaque in patients with CP during a short 7-d follow-up. Essential-oils mouthwash significantly reduced the levels of *Fn* and total bacteria in the CP patients, and this reduction may explain its effectiveness in reducing supragingival plaque and gingivitis. No significant changes in bacteria were observed in periodontally healthy participants. This was a short-term 7-d preliminary study; therefore, the successful reduction of microbes may be transitory. Longer follow-up studies are essential to assess whether, and to what extent, this initial beneficial microbiological effect of SRP and essential-oils mouthwash is sustained. Overall, the results show that SRP and essential-oils mouthwash have beneficial effects in reducing bacterial levels. Our understanding of the microbiological effects of SRP and essential-oils mouthwash sheds light on oral health and provides a scientific basis for recommending professional dental treatment and the use of the mouthwash in addition to daily self-performed oral hygiene in Chinese adults with periodontitis.

Compliance with ethics guidelines

Jia-yan HE, Gang-gang QI, Wu-jing HUANG, Xu-dong SUN, Yu TONG, Chun-mei PENG, Xue-ping ZHOU, and Hui CHEN declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all patients for being included in the study.

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