Prevention and therapy of periodontal diseases and oral malodour

*Brush, rinse and cool*

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Prebrushing rinse with water on plaque removal: a split-mouth design

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Abstract

**Aim**
The aim was to evaluate if there is an additional beneficial effect on dental plaque removal of rinsing the oral cavity with water before toothbrushing.

**Methods**
In total 48 non-dental, systemically healthy participants ≥18 years were included in this randomized controlled clinical trial using a split-mouth design. The participants were requested to refrain from any form of oral hygiene for 48 hours. First dental plaque scores (PI) were assessed full-mouth. Two randomly assigned contra-lateral quadrants were brushed. Next the participant rinsed for 1 minute with 15 ml water. Subsequently, the opposite two contra-lateral quadrants were brushed. Brushing was performed without toothpaste. Subsequently the second full-mouth PI assessment was performed. The brushing and rinsing procedure was done under supervision and brushing time was tracked by a timer, each quadrant was brushed for 30 s. For the buccal, lingual, and approximal surfaces and tooth type a sub-analysis was performed.

**Results**
At baseline there was no statistically significantly difference between the two sets of contra-lateral quadrants. When a water rinse was used before toothbrushing the PI-score was reduced by 58%. If water rinse was used post-brushing the PI-score reduced by 57%. The difference of 0.04 in mean plaque index score reduction between the two brushing regimens was not significant (P = 0.162).

**Conclusion**
When a 2 minutes brushing exercise was performed, on average more than 55% dental plaque was removed. Pre-rinsing with water did not contribute significantly to toothbrush efficacy.
Introduction

In a healthy situation, dental plaque and adjacent tissues maintain a delicate balance. When healthy dental plaque changes into a pathogenic bio-film, this can initiate the periodontal disease process. Toothbrushing is today the most widespread mechanical means of controlling plaque at home (1) and the cornerstone to prevent and control periodontal diseases (2,3). Supragingival plaque is exposed to saliva and to the natural self-cleansing mechanisms existing in the oral cavity. However, although such mechanisms may eliminate food debris, they do not adequately remove dental plaque (2,4). With respect to daily home care the American Dental Association recommends to brush at least twice a day, for 2 min. After tooth brushing, it is common practice to rinse the mouth with tap water with the intention to remove dentifrice remnants and loosened plaque (5). Other researchers have proposed to rinse before brushing in order to hydrate the biofilm, reduce adherence and render plaque more readily detached by mechanical cleaning methods. Changing oral care habits by introducing a prebrushing rinse would mean a change in the world of oral home care (6). A systematic review evaluated the effectiveness of a marketed prebrushing rinse in plaque removal (7). The review authors concluded that the prebrushing rinse appears to offer some benefit in plaque reductions. This specific mouthwash was compared to control groups which were rinsing, with either a placebo or water. A stepwise toothbrush exercise showed that prerinsing with water prior to brushing provided additional plaque removal (8). However, no comparison to regular toothbrushing was performed. The only study that did compare a prebrushing rinse with water to regular toothbrushing was the study by O’Mullane et al. (9). They did, however, not evaluate the effect of a brushing exercise per sé but assessed plaque scores over time with different interventions. Although not significant, the numerical data may be considered indicative of a difference between no rinse and prerinsing with tap water. The purpose of the present study was to compare more specifically Prerinsing with water prior to toothbrushing with common daily toothbrushing that is followed by rinsing with water afterwards.

Materials and methods

Ethical procedures
The study followed instructions based on the Helsinki principles (2013), approximating Good Clinical Practice guidelines and held the approval of the medical ethical committee of the Amsterdam Medical Centre #2014_118. The study was also registered at the Dutch Trial Register (#NTR4604). The study took place in the time period of June until September 2014 at the Academic Centre for Dentistry of Amsterdam (ACTA), the Netherlands, Department of Periodontology. Before enrolment, all volunteers were given verbal and written instructions as well as a description of the aim, rationale and duration of study participation. The investigator explained the details of the trial and potential risk involved. All eligible subjects that agreed to participate signed an informed consent form prior to the study procedures, in addition they were informed that they were free to withdraw from the trial at any time.
Sample size
Sample size calculations were performed using the PS Power and Sample Size Program (10). Earlier data indicated that the difference in the response of the primary outcome in matched pairs is normally distributed with standard deviation 0.30 [pooled estimate out of Van der Weijden et al. (11–13)]. If the true difference in the mean response of matched pairs is 0.14, 38 participants were needed to be able to reject the null hypothesis that the response difference is zero with probability a (power) 0.8. The Type I error probability associated with this test of the null hypothesis was 0.05. Additional participants were entered to compensate for potential dropout.

Screening and inclusion
The participants were non-dental students from different universities and colleges in and around Amsterdam who were invited by e-mail and flyer advertisement. The first selection was performed by a general telephone screening. Additionally, in the clinic the participants were screened by a dental hygienist (MPCL). Inclusion criteria were: ≥18 years old, classified as systemically healthy as assessed by the medical questionnaire, periodontally healthy as assessed by scoring the DPSI ≤3 min (14, 15), ≥5 teeth per quadrant and right-handed brushers. Excluded were those that presented with an orthodontic appliance or a removable (partial) denture, overt caries, any pathological alterations of the oral mucosa, pregnancy and use of medications within 2 weeks before the appointment including antibiotics or chronic use of non-steroidal anti-inflammatory drugs (excluding birth control pills).

Design
This study had a single-(examiner) blind design using a split mouth model where contralateral quadrants were randomly assigned to a treatment (16). The study coordinator (EVDS) supervised the assigned brushing procedures.

Randomization and blinding
Every participant received a unique subject identification number. No stratification was applied. Randomization was performed using true random numbers, which were generated by sampling and processing a source of atmospheric noise (17). The randomization code was kept in a sealed envelope in the investigator site file and was only accessible by the coordinator who was therefore responsible for allocation concealment. Participants were instructed not to reveal their intervention in any way to the clinical examiner. Records of earlier examinations were not available to the examiner at the time of re-examination.

Primary outcome
The main study parameter was the level of full-mouth dental plaque as measured by the Silness & Loe Plaque index (PI) (18) and modified as described by Van der Weijden et al. (11). Where 0 = no plaque, 1 = a film of plaque adhering to the free gingival margin and adjacent area of the tooth 2 = moderate accumulation of soft deposits on the tooth and gingival margin that can be seen with the naked eye, 3 = abundance of soft matter on the tooth and gingi-
val margin (19). Throughout the study examinations were carried out by one and the same experienced examiner (NLHH) under the same conditions. At the time of examination, the examiner was unaware of the randomization scheme.

**Study procedure**
A 48-h non-brushing period was used in order to accumulate an adequate amount of plaque to assess the incremental effect of a single brushing exercise (20). An SMS (Short Message Service) was sent to remind each participant before the visit. First, a full-mouth assessment of dental plaque score was performed. Then the participants went to a separate room, away from the examiner. The intervention was performed under the supervision of the study coordinator. Before the brushing exercise a short verbal instruction was given and no mirror was available. Two randomly assigned contra-lateral quadrants were brushed without dentifrice using a new and prewetted toothbrush (Oral-B indicator P35 soft). Each side of the quadrants was brushed for 15 s, in total 30 s per quadrant. If necessary the participants were able to expectorate saliva after each period of 15 s. Next, the participants rinsed with 15 ml mineral water (Christaline SAINTE-SOPHIE bottled Spring water) for 1 min. The instruction was to take the total amount of water that was inside the cup into their mouth and rinse laboriously without swallowing. Additional subsequent rinsing was not allowed. Next the opposing two quadrants were brushed using a new and prewetted toothbrush following the same procedure as previously described. The supervised brushing and rinsing procedure was tracked by a timer. Following the brushing–rinsing–brushing exercise, a full-mouth plaque assessment was performed to obtain the post-brushing plaque scores. Additionally, the participant completed a questionnaire designed to evaluate their opinion. Their perception was questioned concerning the 48-h non-brushing period, the toothbrush and the procedure used. The participants placed a vertical mark on a 10-cm-long uncalibrated line. The VAS-score line had two extremes at either end of the line with the negative extreme on the left side. The investigator then measured the distance along the line from the left extreme to the marking made by the participant (21). At the end of the study, participants received monetary compensation for their contribution.

**Statistical analysis**
The statistical analysis using SPSS software (Statistical Package for Social Sciences, version 20.0 for Windows) was performed before the randomization code was broken. The primary analysis was the overall dental plaque measurements. The mean and standard deviations were calculated for all sites for each participant, and in addition, the mean incremental difference and proportional change were computed. For a sub-analysis, all vestibular sites, all lingual sites and all approximal sites were calculated. The central incisors were excluded from the measurement to avoid crossover effects of brushing the adjacent teeth. The total mean and variation of the dental plaque scores were analysed for each participant and subsequently by group. Data were tested for normal distribution by the Kolmogorov–Smirnov test and found to correspond with the normal distribution assumption. Parametric statistical technique by the use of a paired T-test was applied. Values of P < 0.05 were accepted as statistically significant. The mean scores and standard deviation
on the VAS questions were calculated. As the VAS questions were descriptive and did not differentiate between groups, no statistical analyses were performed concerning these data.

Results

In total, 50 participants were assessed for eligibility (Fig. 1) (22). Two participants were excluded due to the presence of overt caries and periodontitis. In total, 48 participants were included. The mean age of the 48 participants was 22.5 (2.81) with a range of 20–34. In total, there were more female participants in this clinical trial (N = 28, 58%) than men (N = 20, 42%). No adverse events were reported.

Primary outcome

Table 1 shows the mean and standard deviation of the total dental plaque scores. The split-mouth design implicates that each participant contributed with 2 baseline and 2 post-brushing values. The rinsing and brushing procedure of this study was carefully standardized and controlled. For the post-brushing rinse group, the baseline plaque score was 1.69 (0.32) and for the prebrushing rinse group 1.66 (0.33) which was not significantly different. The post-brushing rinse assessment revealed a reduction in plaque scores of 0.95 (0.20) in the prerinse group compared to a reduction of 0.91 (0.21) in the post-rinse group. Both groups showed a significant reduction in dental plaque. However, there was no statistically significantly difference between the groups on the end scores (P = 0.935). The difference of 0.04 in mean plaque index score reduction between the two brushing regimens was not significant (P = 0.162). When the mouth was rinsed with water before brushing, plaque scores reduced by 58% as compared to a 57% plaque score reduction with rinsing after brushing (P = 0.722).

Subanalysis

Subanalysis (Table 2) was performed for the various surfaces, analysing buccal sites, lingual sites or approximal sites separately. The baseline approximal scores between interventions were not balanced well (P = 0.027). Regarding the lingual sites, 68% and 64% plaque score reduction was found for prebrushing or post-brushing water rinsing, respectively, which was close to being significant (P = 0.049). No further statistical differences were found for pre- and post-brushing rinse data.

Secondary outcome

In total, six questions were asked after the assessment (Table 3). The participants felt rather uncomfortable to refrain 48 h of any oral hygiene before the appointment (VAS is 2.57). The participants felt that the toothbrush bristles are neither too hard or too soft (4.87) nor felt pleased. They scored the use of this manual toothbrush with a 6.04, which is close to the mean value of 5. A brushing exercise without the use of dentifrice was judged as unpleasant (3.07). Prerinsing with water was felt moderately pleasant (6.66), and the 1 min rinsing duration seems appropriate (6.18).
**Figure 1** Flow chart depicting subject enrollment and measurement \([22]\).

* these quadrants are used as post brushing rinse
‡ these quadrants are pre brushed rinse
Q = Quadrant

Recruitment

Informed consent + screening (DPSI)

Assessed for eligibility \((N=50)\)

Excluded \((N=2)\):
- Overt caries \((N=1)\)
- Periodontitis \((N=1)\)

Refraired of 48h brushing

Baseline plaque score

Randomized Split-mouth \((N=48)\)

\(N=24\)

Supervised Brushing* 
Q2+Q4

Supervised Rinsing 
15 ml water

\(N=24\)

Supervised Brushing* 
Q1+Q3

Supervised Rinsing 
15 ml water

End plaque score

Questionnaire

Analyzed \((N=48)\)
The aim of the present study was to compare rinsing with water prior to toothbrushing with toothbrushing followed by rinsing on the removal of dental plaque. The plaque scores were reduced by more than 55%, whereas no difference was obtained between the two interventions. Similar results were also found for the subanalysis on various surfaces.

**Study design used**

Bentley and Disney (16) evaluated the comparison of partial and full-mouth scores of plaque and gingivitis without compromising study quality. Their analyses showed that all-teeth, the Ramfjord teeth, half mouth and molars/anteriors revealed a similar pattern as whole-mouth and proximal areas. The present study used an examiner blind split-mouth model with randomly assigned contra-lateral quadrants for each allocation. The advantage of this design is that it reduces interindividual variability from the estimates of the treatment effect, because participants acted as their own control (23). Also the impact of the ‘Hawthorne effect’ of present study would have impacted both interventions similarly because the same operator performs both interventions in the same oral cavity. The split-mouth randomization was applied in the current design in such a manner that all participants contributed with two quadrants to the prebrushing rinse and with two to the post-brushing rinse data. The rationale for using contra-lateral quadrants is that the skills of the individual using the brush may affect plaque removal. To rule out the influence of dexterity, contra-lateral quadrants were randomly assigned. This has been used in previous brushing evaluations also using the same plaque index (24,25). Additionally, only right-handed brushers were included, because a previous investigation showed a significant difference between right- and left-handed participants (13).

**Rinsing procedure used**

The results of a meta-analysis have demonstrated that a commercially available pre-brushing rinse has a positive effect on reducing plaque score levels (7). The present study evaluated solely the additional effect of water as a prebrushing rinse. Based on the data of a previous study (26), a rinse volume of 15 ml was used for this experiment. On a 0–10 VAS scale (extremely unpleasant to very pleasant), participants felt that rising with water was moderately pleasant with a mean VAS score of 6.66.

**Table 1** Overall dental plaque scores on baseline and end presented by mean and SD (standard deviation) when post- and pre-brushing rinse with water was performed.

<table>
<thead>
<tr>
<th>N=48</th>
<th>Post-brushing rinse</th>
<th>Pre-brushing rinse</th>
<th>Statistical analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.69 (0.32)</td>
<td>1.66 (0.33)</td>
<td>0.095</td>
</tr>
<tr>
<td>End</td>
<td>0.75 (0.34)</td>
<td>0.76 (0.33)</td>
<td>0.935</td>
</tr>
<tr>
<td>Incremental difference</td>
<td>0.95 (0.20)</td>
<td>0.91 (0.21)</td>
<td>0.162</td>
</tr>
<tr>
<td>% Reduction</td>
<td>58% (15)</td>
<td>57% (15)</td>
<td>0.722</td>
</tr>
</tbody>
</table>

* Paired sample T-test
Rinsing duration has also been evaluated in previous work. Paraskevas et al. (27) showed that the intra-oral spread of the mouthrinses in relation to the rinsing periods of 15, 30 and 60 s. Rinsing with 30 s was sufficient to allow all plaque-covered surfaces of the dentition to come into contact with the solution. To be on the safe side regarding spread and optimal wetting of the dental plaque for the present study, 1 min prerinsing duration was used. The participants evaluated this duration on a scale from too short up to too long as 6.18. This is just above the neutral score of 5.00 of being neither too short nor too long.

**Brushing procedure used**

Recently, in a metareview concerning the evidence as presented in systematic reviews, the efficacy of available home-care toothbrush regimens for mechanical plaque removal on plaque and gingivitis in adults was evaluated. The conclusion was that tooth brushing is effective in reducing levels of dental plaque (28). The efficacy in plaque removal following a brushing exercise is a reduction from baseline plaque scores of 42% between pre- and post-brushing on average, with a variation of 30–53% dependent on the plaque index used. This variation based on plaque indices is also supported by the results of a study by Binney et al. (8) showing that the overall effect of brushing upon plaque reduction with the Quigley & Hein Plaque Index (29) was 27%, whereas in the same participant, a 64% reduction in ‘plaque area’ scores was observed. The results of the present study have shown that using a manual toothbrush and assessing plaque according to Silness & Löe plaque index 57% and 58% reductions are observed for the pre- and post-brushing water rinse, respectively. An explanation for more plaque removal in the present study as compared to Slot & Van der Weijden (28) may in part be explained by the Silness & Löe plaque index used (18). Even a difference in outcomes between the Turesky (30) and Lobene (31) modification of the Quigley & Hein Plaque Index (29) has been shown (20). Also, the supervised brushing may have improved efficacy. This supervision is often performed in research to make sure that the

<table>
<thead>
<tr>
<th>Sites</th>
<th>N=48</th>
<th>Post-brushing rinse</th>
<th>Pre-brushing rinse</th>
<th>Statistical analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.49 (0.58)</td>
<td>1.48 (0.55)</td>
<td>0.957</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>0.30 (0.27)</td>
<td>0.38 (0.29)</td>
<td>0.121</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>1.18 (0.51)</td>
<td>1.11 (0.42)</td>
<td>0.294</td>
<td></td>
</tr>
<tr>
<td>Lingual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.53 (0.45)</td>
<td>1.45 (0.46)</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>0.49 (0.33)</td>
<td>0.52 (0.36)</td>
<td>0.531</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>1.04 (0.36)</td>
<td>0.93 (0.36)</td>
<td>0.049**</td>
<td></td>
</tr>
<tr>
<td>Approximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.79 (0.29)</td>
<td>1.74 (0.31)</td>
<td>0.027**</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>0.91 (0.43)</td>
<td>0.89 (0.40)</td>
<td>0.506</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.88 (0.24)</td>
<td>0.85 (0.23)</td>
<td>0.218</td>
<td></td>
</tr>
</tbody>
</table>

* Paired sample T-test
** Significant difference between the groups (P<0.05)
study procedures including brushing duration are performed according to the protocol.

Finally, also the non-oral hygiene period prior to the clinical assessment may have affected
the plaque score reduction. The longer the plaque could accumulate freely on the dental
surfaces, the higher the plaque score reduction. An explanation could be that if there is
more plaque present on the tooth surface, there is simply more plaque to remove (20).

In the present study, no dentifrice was used during the brushing procedure. Based on the
VAS questionnaire regarding this aspect, the participants’ perception was that this was
not considered to be pleasant. Already, half a century ago, this was also evaluated. The ge-
eral reactions of participants who used no dentifrice were most unfavourable (32). The
use of a dentifrice is in general stimulated by many factors such as pleasantness of flavour
and aftertaste, foaming quality, mouth feel during application, removal of tooth stain, fee-
ling of freshness and cleanliness (33). This also contributes to confidence of not offending
others by ‘bad breath’ (34). However, the effect of dentifrice on plaque removal compared
to brushing with only water showed that the reduction in plaque index by brushing with a
dentifrice was less marked than brushing with water (28). Already, in 1967, Bergenholtz et
al. (35) reported that brushing without dentifrice was more efficient than similar brushing
with dentifrice. The findings in Binney et al. (8) demonstrated that none of the commercial
dentifrices tested possessed plaque removal properties of clinical significance and cer-
tainly little greater than what might be achieved with water or a slurry of toothpaste.

**Post-brushing rinsing**

Rinsing with water after brushing with fluoride toothpaste can reduce the benefit of
fluoride toothpaste. Davies et al. (36) recommend discouraging rinsing with water after
brushing. As an alternative, a fluoride mouthrinse can be used after brushing. Because
most people are used to rinsing after brushing, this will certainly help compliance. This
recommendation could have a beneficial effect for caries control.

<table>
<thead>
<tr>
<th>N=48</th>
<th>Extreme</th>
<th>From</th>
<th>To</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I think the bristles are...</td>
<td>Very hard</td>
<td>Very soft</td>
<td>4.87 (1.65)</td>
</tr>
<tr>
<td></td>
<td>I think brushing without a dentifrice is...</td>
<td>Unpleasant</td>
<td>Pleasant</td>
<td>3.07 (2.45)</td>
</tr>
<tr>
<td></td>
<td>I think rinsing with water is...</td>
<td>Unpleasant</td>
<td>Pleasant</td>
<td>6.66 (1.68)</td>
</tr>
<tr>
<td></td>
<td>I think the time of rinsing [1 minute] is...</td>
<td>Too short</td>
<td>Too long</td>
<td>6.18 (1.35)</td>
</tr>
</tbody>
</table>

**Table 3** Questionnaire related to the self-perception of the participants using a Visual Analogue Scale (VAS)
presented as mean and standard deviation (SD) with negative extremes on the left and positive extremes on the
right(from 0 to 10).
Limitation and directions for further research

- The current study presents the results of young healthy adults. Older adults with wider interdental spaces might show different results.
- The effect of post-brushing rinsing with water may have an additional effect on plaque removal. This aspect has not been studied before so that the potential impact on outcomes is unclear.
- In the present study, we used a new- and prewetted toothbrush for each intervention. O’Hehir and Suvan (37) suggested that lingual surfaces should be dry-brushed first. In a practice-based evaluation, they noted significant improvements. Both these aspects could be the topic of future evaluations.

Conclusion

When a 2 min brushing exercise was performed, plaque score reduced by more than 55%. The study does not support that the use of a prebrushing rinse provides a greater effect than mechanical plaque removal by a manual toothbrush.

Acknowledgements

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest. Authors and their research group at ACTA have formerly received external advisor fees, lecturer fees and/or research grants from toothbrush manufacturers. Among these were Braun AG, Colgate, Dentaid, GABA, Lactona, Oral-B, Philips, Procter & Gamble, Sara Lee, Sunstar and Unilever.

Authors contributions

Conception or design of the study: EVDS, DES, NLHH, GAW
Analysis and/or interpretation of the data: EVDS, DES, MPCVL, GAW,
Drafted the manuscript: EVDS
Critically revised the manuscript: DES, NLHH, MPCVL, GAW
All authors gave their final approval and agreed to be accountable for all aspects of the work
References


