Prevention and therapy of periodontal diseases and oral malodour
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Citation for published version (APA):

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General discussion, summary and conclusions
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Periodontitis is one of the two most important oral diseases that contributes to the global burden of chronic disease (1), the prevalence of which increases with age (2) and represents a significant burden to public health (3). Maintaining a healthy oral cavity involves the prevention and therapy of gingival inflammation and dental caries (4,5). Oral malodour may have a negative impact upon an individual’s quality of life, confidence, speech, oral health and overall well-being (3,6,7). The reported prevalence of oral malodour ranges widely (8). This thesis aims to evaluate different uses of water or mouthwash in relation to specific brushing and rinsing instructions as well as the use of cooling solutions. Measures related to the prevention and therapy of periodontal diseases and oral malodour are the result of this evaluation.

Oral hygiene

The basics

To prevent and control periodontal diseases, effective plaque control is necessary, for which toothbrushing is universally recommended (3). Toothbrushing effectively reduces levels of dental plaque (9) and thus, the general recommendation is to brush twice a day with a fluoridated dentifrice for at least two minutes each time (3). The use of interdental cleaning devices is necessary to maintain interproximal gingival health. Evidence has further indicated that the interdental brush is an effective method for interproximal plaque removal (3). The ideal brushing manner is the one that effectively removes all plaque in the least possible period of time and with no damage to the tissues in the oral cavity (10). In general, brushing methods can be classified based on the position and movement of the toothbrush. Recommendations on toothbrushing techniques appear to differ with respect to, for instance, how many times a day individuals should brush their teeth and for how long. No particular brushing technique has been found to be clearly superior, but the most common manner recommended is the (modified) bass technique (11).

Brushing sequence

The lingual surfaces of the mandibular teeth usually harbour an abundance of plaque and are difficult to clean (12). Therefore, a patient should pay special attention to these surfaces by brushing them first, as has been proposed by dental care professionals (DCPs) (13). The randomised controlled clinical trial from Chapter 2 that uses a split-mouth design focused on a specific brushing sequence. Before the visit, the participants were asked to refrain from any oral hygiene procedure for 48 hours. Subsequently, a full mouth plaque score was performed. Two randomly chosen contra-lateral quadrants were used to start brushing from the lingual surfaces first. The opposing two quadrants were used to start brushing from the buccal surfaces. After the brushing exercise was completed, post-brushing scores were assessed. Beginning with the lingual surface of the lower jaw resulted in a 55% reduction of plaque scores, compared to 58% when the brushing exercise began with the buccal surface and the scores were not statistically different. The difference in mean plaque score reduction between brushing sequences was 0.04 and also, not significant. Furthermore,
none of the sub-analyses for the buccal, lingual and approximal surfaces revealed any significant difference. Within the limitations of this study, a recommendation to start toothbrushing from the lingual aspect does not receive scientific support based on the outcome of the experiment.

**Pre-brushing rinse**

It has also been suggested that when a pre-brushing rinse is used, dental plaque might be more easily removed with a toothbrush. The aim of Chapter 3 was to evaluate whether rinsing the oral cavity with water before toothbrushing results in an additional beneficial effect on dental plaque removal. A similar research design was used, as described within the previous Chapter (2). The brushing and rinsing procedure was supervised and brushing time was tracked by a timer. The participants brushed each quadrant for 30 seconds. When a rinse with 15 ml water for 1 min was performed before manual toothbrushing, plaque scores reduced by 58%, compared to 57% when participants rinsed with water after brushing. The difference of 0.04 in the mean plaque index score reduction between the two brushing regimens was not significant. Furthermore, sub-analyses for the buccal, lingual and approximal surfaces revealed no significant differences. The results, as presented within Chapter 3, do not support that rinsing with water before brushing results in a greater effect than mechanical plaque removal by a manual toothbrush.

**Dry brushing**

Another professional belief is that dry toothbrushing improves the perception of ‘smooth’ for those surfaces which are effectively cleaned after brushing. Toothpaste may interfere with the perception of cleanliness due to the flavour and wetting ingredients used (13). A clinical trial has evaluated first brushing with a dry toothbrush until all teeth felt clean and tasted clean. In a second step, dentifrice was added and the teeth were brushed once more to provide the necessary fluoride (13,14). Because this trial lacked a control group, no high quality research is available to support that dry brushing is indeed more effective. In Chapters 2 and 3, two similar single-brushing exercises were performed, one of which included brushing with a pre-wetted toothbrush (Chapter 3) and the other involved brushing with a dry toothbrush (Chapter 2). Because these experiments were performed under the same conditions with the same participants, the same study design and the same examiners, a secondary analysis could be performed regarding dry brushing (Chapter 4). The dental plaque score reduction following brushing with a dry toothbrush was 58%, compared to the 57% result of using a pre-wetted toothbrush. The mean difference in plaque index score reduction (0.09) between a dry and a pre-wetted toothbrush was not significant. Pre-wetting the participants’ toothbrushes appeared to have no influence on the perception of toothbrush filament stiffness nor on the perception of cleaning capability. However, brushing without a dentifrice was judged as unpleasant. In summary, the three specific variations in toothbrushing instruction, as provided by DCPs to improve brushing efficacy, evaluated within this thesis (Chapters 2, 3 and 4) appear to be based on empirical evidence and are not supported by scientific evidence. Consequently, these presumptions should not be adapted as general advice when DCPs instruct their patients on toothbrushing methods.
**Instruction**

Substantial evidence has indicated that toothbrushing and other mechanical cleansing procedures can reliably control plaque, provided that cleaning is sufficiently thorough and performed at appropriate intervals (9). It is recommended that oral hygiene instructions be given during a series of appointments, allowing the patient to receive immediate feedback and improve the patient’s oral hygiene. Toothbrushing methods must be implemented according to the patient’s needs (15). Based on a systematic review, the effects of oral hygiene motivation, information and instruction can continue even after six months (16). The design of toothbrushes or a specific toothbrushing method are likely of secondary importance for each individual (17). The individual motivation, manual skills, complexity of the dentition and self-consciousness all contribute to an optimal effect (18). Thus, it is considered important to make the individual aware of their own ability to maintain proper dental health with minimal participation of the DCPs, which could be limited to a mere advising and encouraging role (19). However, changing health behaviour in case of persistent noncompliance is probably one of the most challenging goals for DCPs (20). The technology of a smart digital toothbrush monitoring and training system was found to improve individuals’ brushing technique, leading to enhance oral hygiene. Eventually, the focus should be on the systematics during toothbrushing so that all surfaces are reached, rather than on changing one’s toothbrushing technique (18).

**Toothbrushing and water**

The toothbrush filaments used in the studies presented in Chapters 2, 3 and 4 were soft. The literature is inconclusive concerning the benefit of toothbrushing with softer filaments. Indeed, it has been suggested that toothbrushes with medium or hard filaments are able to better remove plaque. However, these bristles can cause more gingival abrasions than toothbrushes with soft filaments (21,22). Moreover, pre-wetting a toothbrush in warm water leads to a loss in mechanical resilience of the filaments (23), since the temperature of the water can adapt toothbrush bristle morphology (24). It has further been suggested that patients can soften the toothbrush in hot water for those areas where gingival surgery was performed (25). This, however, has not been supported by any study.

**Oral malodour**

**Measurements**

For the unpleasant smell on an individual’s breath (26), toothbrushing alone is not enough to reduce oral malodour (27,28). To diagnose oral malodour, the organoleptic score (ORG) is considered as the gold standard (29). Specific apparatuses objectively assess the levels of volatile sulphur compounds (VSCs). VSCs are considered to be the most significant gases with regard to oral malodour: hydrogen sulphide (H₂S), methyl mercaptan (CH₃SH) and dimethyl sulphide ((CH₃)₂S)). Of these three compounds, H₂S and CH₃SH, are the main contributors (30). Based on the data currently available within the literature, two devices for the detection of VSC can be recommended for use in dental care practices (31). A portable sulphide monitor, the Halimeter®, has acceptable sensitivity and specificity (26). The OralChroma™ is also suitable in research environments since it is more specific for three separate gases (32).
Both apparatus exhibit an acceptable correlation with scores from a calibrated odour judges and appear to be easy to use under the conditions found in a regular dental practice (31).

Water
Life style rules regarding oral malodour studies often request that participants abstain from eating and drinking (even water) for a specified number of hours before the assessment. The impact of frequently drinking water on oral malodour was evaluated in Chinese adults. It appeared that those without oral malodour drank water more frequently (5 times or more per day) than the oral malodour group (33). Furthermore, within the laymen’s literature, it has been suggested that the use of water could positively affect morning bad breath (MBB). This was the subject of the research in Chapter 5. Participants were randomly divided into two groups: rinsing with water for 30 seconds or drinking a glass of water within 30 seconds. Assessments were carried out during a morning appointment, between 7:30 am and 12:00 pm. The participants had fasted overnight. As primary outcome parameters, the ORG was assessed. The output of the apparatuses that evaluate VSCs, the OralChroma™ and the Halimeter® were the secondary outcome parameters. While rinsing with water and drinking a glass of water had a statistically significant effect on the MBB parameters, no significant difference was found between the two.

Mouthwash
Mouthwash is an ideal vehicle for the active ingredients that impact oral health and prevent disease (34,35,36). Different types of mouthwash reduce oral malodour (37). Thus far, one study has evaluated the additional effects on of mouthwash tongue cleaning over two week period. However, no additional effects on oral malodour were found (38-41). Chapter 6 evaluates a toothbrushing regimen that consists of a tooth/tongue gel, tongue cleaner and mouth rinse, based on the ORG, VSCs and tongue surface appearance scores. During the 3 week, parallel, single-blind, randomised, controlled clinical trial, the test group regimen used a tongue cleaner, a tooth/tongue gel and a mouth rinse that contained a combination of amine fluoride, stannous fluoride and zinc lactate to counteract oral malodour. The control group regimen consisted only of a standard fluoride dentifrice. A significant effect on MBB was observed after 24 hours for most of the parameters in favour of the test regimen. On day 21, the decrease in H₂S and the Halimeter® outcomes were maintained for the test group regimen. A significant increase in tongue surface discoloration was observed in the test regimen. Participants’ self-perceptions also indicate that their breath felt more fresh when they woke up. No effect was observed for the primary ORG outcome parameter. A surrogate method to screen for oral malodour is described within the literature as ‘turbidity’ of the mouth-rinsed water (42). It is defined as ‘an expression of optical property that causes light to be scattered and absorbed, rather than transmitted in straight lines, through the sample’. This could be a less expensive method. However, the clinical relevance still needs to be addressed. Many factors, including dental status, periodontal condition, oral hygiene and flow rate of saliva, affect oral malodour. Similarly, the turbidity of mouth-rinsed water could be influenced by these factors (42). Furthermore, self-estimation can be used to judge one’s own oral malodour, which was found to be significantly correlated with H₂S and CH₃SH (43).
Tongue surface characteristics

Tongue surface characteristics are also of interest with regard to oral malodour. Tongue coating is closely associated with clinical oral malodour, whereas a high plaque index is closely associated with perceived oral malodour (44). Periodontal diseases can be an additional, but less important, cause of oral malodour as not all periodontally affected patients will have oral malodor. Moreover, even periodontally healthy patients can present with malodour. In a small number of patients, gingivitis or periodontitis can be the single cause of oral malodour (45). The main etiology of oral malodour in young people is tongue coating and in the older generation it is a combination of periodontal diseases and tongue coating (46). Several methods have been used to assess the presence of tongue coating. One approach is to carefully remove the tongue coating with a tongue scraper and either estimate the wet weight (47) or use an ordinal index to assess the amount (48,49). The tongue surface can be differentiated by distribution area (46), colour and quality (50). Other methods have visually divided the dorsum of the tongue into different areas and, per area, the tongue surface discoloration and tongue coating thickness are given a score (51). The tongue surface scores in Chapters 5 and 6 were assessed according to the Gomez index (52). The dorsum of the tongue was divided into nine areas and the tongue’s surfaces were scored according to discoloration and coating thickness. Tongue surface discoloration was scored on a scale from 0 to 4, e.g. score 0=pink, and score 4=black. The tongue coating thickness ranges from 0 to 2, e.g. score 0=no coating, and score 2=heavy-thick coating. On the posterior- and mid-dorsal regions of the tongue, a discoloration score of 2 was primarily observed. This was similar for the tongue coating thickness.

In a clinical trial, the test group that used products containing chlorhexidine (CHX), cetylpyridinium chloride and zinc-lactate exhibited significantly higher tongue surface discoloration compared to the placebo group (51). These results are in accordance with the findings in Chapter 6, where the tongue surface exhibited a significant increase discoloration over time. The presence of a tongue surface coating does not necessarily lead to oral malodour and moreover, reduction of the VSC production can be achieved in the presence of tongue coating (51). However, the tongue surface is the primary strong odour-forming site in the mouth and the removal of tongue coating has the potential to reduce VSCs. The evidence for the beneficial effect of mechanical tongue cleaning in real oral malodour patients remains weak (8) and cleaning the tongue surface only appears to have a short-lived effect on intraoral malodour (53). On the other hand, a systematic review concluded that nearly all mouthwash containing active ingredients had beneficial effects on reducing oral malodour (37). Therefore, cleaning the tongue and the use of a mouthwash with active ingredients, as a regimen, could have a positive effect on oral malodour. For instance, the combination of a tongue brush and an antibacterial tongue spray containing 0.09% cetylpyridinium chloride and 0.7% zinc gluconate, was able to provide more than 6 hours of fresh breath after a single use (54). Another study revealed that toothbrushing combined with tongue cleaning results in lower levels of VSC and tongue coating compared to only toothbrushing (55). Additionally, a regimen of toothbrushing, mouthwash and tongue cleaning exhibited the most reduction in oral malodour (27). However following the use of a mouthwash containing zinc and CHX a decrease in effect oral malodour was observed to which, tongue cleaning did not provide an additional effect (38,39).
The impact
Several studies have indicated that, given the potential social consequences of oral malodour, it is vital for DCPs to make patients aware of the presence of oral malodour. Indeed, DCPs should be prepared to practice in a culturally diverse environment as well as in a sensitive and appropriate manner to provide optimal oral health and hygiene care and improve patients’ oral health related quality of life (HRQoL) and well-being (56). The HRQoL or simply, quality of life (QoL), constructs are commonly assessed by patient-reported outcomes (PRO) questionnaires. Oral health-related quality of life (OHRQoL) is ‘the impact of oral disorders on aspects of everyday life that are important to patients and persons, with those impacts being of sufficient magnitude to affect an individual’s perception of their life overall’ (57). Oral malodour is one of the most common complaints from patients in the clinics (33). When OHRQoL was measured using the oral health impact profile (OHIP-14), those with oral malodour scored lower (33). It has even been suggested that an effective oral health behaviour may play an important role in oral malodour and influence an individual’s self-perceived OHRQoL (56). Patients with oral malodour may even experience psychological discomfort and disability (33). In both chapters on MBB (Chapters 5 and 6) participants’ self-perception was evaluated using a visual analogue scale (VAS) questionnaire. The VAS has been extensively used in the evaluation of health related QoL (58). Although it is difficult to use objective criteria for a subjective self-perception (59), the VAS is considered an established tool that is easy to use and has been found to differentiate between groups (60-64). After the intervention, the rinse group in Chapter 5 perceived their own breath as significantly more fresh than the drinking group. Furthermore, in Chapter 6, the improvement of the perceived freshness of breath upon wakening, for the test participants who were assigned to the regimen that included mouthwash, was significantly greater than for the control group. Patients with potential oral malodour do not always receive the appropriate care, which may be due to a lack of knowledge of DCPs on this specific outcome (65-67). A possible explanation for this may be reluctance on the part of the DCPs, which seems to interfere with adequate professional care (56). In general, oral malodour may rank behind, since dental caries and periodontal diseases are the reasons why patients visit the DCPs (68).

Periodontal diseases
Chemical anti-plaque agents
Meta-reviews have displayed that home-care regimens for mechanical plaque removal are effective (69,70). Over the past decades, the use of anti-microbial mouthwashes have become customary, following mechanical plaque biofilm control (69). When used as an adjunctive therapy to conventional manual toothbrushing with a fluoridated dentifrice, the use of chemical anti-plaque agents in mouthwash or incorporated into dentifrice, alone or in combination, provides clear and significant improvements in managing gingival inflammation and preventing plaque accumulation (3). There are several systematic reviews concerning ingredients in mouthwash products. Based on these systematic reviews, Chapter 7 presents the results of a meta-review that evaluated the effect of various types of mouthwash on plaque and gingivitis. Evidence suggests that a mouthwash containing CHX is the first choice. The most reliable alternative for plaque control is essential oils (EO).
Regarding gingivitis however, no difference was observed between CHX and EO. Similar results were established by a recent systematic review and meta-analysis, which compared the efficacy of different anti-plaque chemical mouthwash products, in 6-month, home-use, randomised clinical trials, with plaque index changes as outcome parameters. Mouthwash containing EO or CHX exhibited the greatest effect on plaque index score reduction (71). In an earlier review, the same research group also demonstrated a positive effect on parameters of gingival health (72).

**Cooling solution**

Additionally, mouthwash with active ingredients can also be used during non-surgical periodontal therapy as a cooling solution, such as in combination with ultrasonic devices. Mouthwash can be useful, for a pre-procedural rinse, in reducing the bacterial loading within the oral cavity (73-75). Furthermore, mouthwash containing additional active ingredients can be used as a cooling solution following non-surgical therapy with ultrasonic devices. Chapter 8 presents the results of a systematic review on the effect of clinical parameters of periodontal inflammation following non-surgical therapy with ultrasonic devices and chemotherapeutic cooling solution. Efficacy on the clinical parameters of periodontal inflammation, such as probing pocket depth (PPD) and clinical attachment level (CAL), was evaluated. The meta-analysis revealed that even when the ultrasonic cooling solution contained adjuvant active ingredients, the difference of means and for CAL were not statistically significant. The sub-analysis further indicated that, in conjunction with povidone-iodine (PVP), a very small gain in CAL may be expected. PVP is mainly used in a 0.5%-1.0% concentration. The included study in Chapter 8 that contributed with a significant effect on the incremental score PPD is do Vole et al. 2016 (76). This study, however, used a much higher percentage of 10% PVP. Another, non-included, study showed that PVP as a cooling solution might enhance PPD reduction in initially deep pockets of non-molar teeth, when the same 10% of PVP was used (77). Their study protocol included that all pockets were refilled with a PVP ointment after instrumentation to obtain a greater contact time. This may have contributed to the study’s positive findings. From the results presented in Chapter 8, it can be concluded that an ultrasonic device with just plain tap water used as a cooling solution is sufficiently effective by itself. Besides as an advantage, water as coolant ensures good visibility for the DCP, because the work field is continuously flushed (78).

**External validity**

External validity refers to the generalizability of the outcome results. Basic aspects of external validity concern whether the subjects, interventions and settings are similar enough to achieve the intended purpose (77, 80). The first key aspect includes the subjects; ‘are the subjects similar to the intended application?’ As previously mentioned, the study population of the current thesis primarily presents clinical outcome results of mainly young healthy adults (Chapters 2, 3, 4, 5 and 6). The age of the participants ranged between 18 and 39 years, the majority of whom are female, systemically healthy and had no periodontal diseases. Furthermore, Chapters 5 and 6 only include non-smokers. The available evidence suggests that oral malodour is common and can affect people of all ages (81).
The second key aspect questions whether ‘the intervention is provided and assessed in a similar way to the intended application?’ The studies presented in Chapters 2, 3 and 4 performed a comparable study design: a single brushing exercise and split-mouth model were used. While Chapter 5 evaluates two different alternative interventions, the study lacked a control that was not affected by an intervention. However, the study showed an effect for both interventions on the organoleptic scores. The examiners could have been influenced by the knowledge that all participants were exposed to an intervention. On the other hand, objective outcomes from the OralChroma™ confirmed the organoleptic scores. Chapter 5 presents a single visit and Chapter 6 examines the follow-up that occurred after 21-days, the included participants were assessed during the same time slot between 7.30 am and 12.00 pm. Both studies required strict life-style rules and the assessments occurred in the same room.

The last key aspect of external validity concerns the setting: ‘is the setting of the study similar enough to the intended application?’ All clinical studies were performed at the department of Periodontology of the Academic Centre of Dentistry Amsterdam. The same trained examiners and organoleptic judge performed the measurements. Furthermore, the brushing procedure and interventions outlined in Chapters 2, 3, 4, 5 and 6 were standardised and controlled. The studies used a typical research set-up to evaluate to what extent an intervention can produce a beneficial outcome under ideal circumstances. The procedures were performed under supervision and were carefully controlled. This aspect could have an impact on the generalisability of the outcome results and may not be representative for the effectiveness of an intervention under ordinary day-to-day circumstances (82). However, an important recommendation is to standardise, within randomised clinical trials, the outcome measurements and exclude possible bias to guarantee the quality of the results (3).

**Conclusion**

Different uses of water in relation to cleaning capability, pre-rinsing and a specific brushing sequence do not significantly contribute to toothbrush efficacy. Water had a positive effect on morning bad breath parameters. In addition to toothbrushing, a regimen of a tooth/tongue gel and mouthwash had a significant overnight effect on morning oral malodour compared to regular oral hygiene. The prolonged effect of the regimen was perceived as fresh by the participants. The adjunctive use of mouthwash containing particularly chlorhexidine or essential oils had an effect on plaque control and managing gingivitis. Moreover, the use of a chemotherapeutic cooling solution in an ultrasonic device on clinical parameters of periodontal inflammation following non-surgical periodontal therapy did not exhibit any additional effects. Thus, plain tap water is an inexpensive and sufficiently effective cooling solution.
References


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75. Logothetis DD, Martínez-Welles JM. Reducing bacteria aerosol contamination with a chlorhexidine gluconate pre-rinse. JADA 1995; 126: 1634-1639.


### List of frequently used abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>ACTA</td>
<td>Academic Centre for Dentistry Amsterdam</td>
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<td>ADA</td>
<td>American Dental Association</td>
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<td>AMC</td>
<td>Academic Medical Centre</td>
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<td>BOP</td>
<td>Bleeding On Probing</td>
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<td>BS</td>
<td>Bleeding Scores</td>
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<td>CAL</td>
<td>Clinical Attachment Loss</td>
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<tr>
<td>CH$_3$SH</td>
<td>Methyl Mercaptan</td>
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<tr>
<td>(CH$_3$)$_2$S</td>
<td>Dimethyl Sulphide</td>
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<td>CHX</td>
<td>Chlorhexidine</td>
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<tr>
<td>CONSORT</td>
<td>Consolidated Standards Of Reporting Trials</td>
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<td>DCP</td>
<td>Dental Care Professionals</td>
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<td>DiffM</td>
<td>Difference in Means</td>
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<td>EO</td>
<td>Essential Oils</td>
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<td>GI</td>
<td>Gingival Index</td>
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<td>GRADE</td>
<td>Grading of Recommendations Assessment, Development and Evaluation</td>
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<td>H$_2$S</td>
<td>Hydrogen Sulphide</td>
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<td>MA</td>
<td>Meta-Analysis</td>
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<tr>
<td>MBB</td>
<td>Morning Bad Breath</td>
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<td>MEDLINE</td>
<td>Medical Literature Analysis and Retrieval System Online</td>
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<td>NVvP</td>
<td>Nederlandse Vereniging voor Parodontologie (Dutch Society of Periodontology)</td>
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<tr>
<td>ORG</td>
<td>Organoleptic Scores</td>
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<td>OZI</td>
<td>Onderzoek instituut ACTA</td>
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<td>PPD</td>
<td>Pocket Probing Depth</td>
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<td>PVP</td>
<td>Povidone-iodine</td>
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<td>PI</td>
<td>Plaque Index</td>
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<td>Plaque Scores</td>
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<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic PubMed</td>
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<tr>
<td>PubMed</td>
<td>Public Medline database</td>
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<tr>
<td>Q&amp;L</td>
<td>Quigley &amp; Hein Index</td>
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<tr>
<td>REC</td>
<td>Recession (distance from the marginal gingiva to the cemento-enamel junction)</td>
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<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>SE</td>
<td>Standard Error</td>
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<tr>
<td>S&amp;L</td>
<td>Silness - Löe Index</td>
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<tr>
<td>SRP</td>
<td>Scaling and Root Planing (non-surgical debridement)</td>
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<td>STROSA</td>
<td>Standardized Reporting Of Secondary Analysis</td>
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<tr>
<td>TIDIER</td>
<td>Template for Intervention Description and Replication</td>
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<tr>
<td>US</td>
<td>Ultrasonic Scaling</td>
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<td>VAS</td>
<td>Visual Analogue Scale</td>
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<td>Volatile Sulphur Compounds</td>
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