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PAPER

Daily reduction of oral malodor with the use of a sonic tongue brush combined with an antibacterial tongue spray in a randomized cross-over clinical investigation

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Abstract

The objective of this clinical investigation was to test the effectiveness on breath odor of a newly designed sonic tongue brush (TongueCare+, TC). It consists of a soft silicone brush optimally designed based on the tongue's anatomy to remove bacterial biofilm from the tongue's complex surface, and it is coupled with a sonic power toothbrush handle. TC was used in combination with an antibacterial tongue spray (BreathRx, BRx) containing 0.09% cetylpyridinium chloride and 0.7% zinc gluconate. A total of 21 participants with oral malodor exceeding the threshold for recognition took part in this cross-over clinical investigation, which consisted of a single use of four treatment arms with one week washout period in between. The treatments consisted of: (1) TC + BRx, (2) TC + water, (3) BRx and (4) water. Malodor levels and bacterial density were monitored up to 6 h by organoleptic scoring and selective plating, respectively. The organoleptic score and bacterial density were significantly lower after using TC + BRx compared to all alternative treatments at all time points. A significant decrease in both parameters was detected after a single use of TC + BRx, from levels characteristic of high oral malodor, to barely noticeable levels after treatment and this was maintained up to 6 h. Moreover, we identified a significant positive correlation between bacterial density and organoleptic score, confirming that bacterial tongue biofilm is the root cause of oral malodor in these subjects. The results of this clinical investigation demonstrated that the combined treatment of a sonic tongue brush with the antibacterial tongue spray is able to deliver more than 6 h of fresh breath following a single use. The clinical investigation was registered at the ISRCTN registry under study identification number ISRCTN38199132.

Introduction

Oral malodor is a widespread problem affecting up to 30% of the global population and 74% consider it as an issue [1]. It has strong social implications for the sufferer and significantly impacts on normal social interactions. Approximately 80% of the cases are of intra-oral origin, caused by volatile sulfur compounds (VSC) produced by bacteria that inhabit the dorsum of the tongue [2, 3]. The tongue has a complex surface structure, with numerous pits, fissures and crypts where anaerobic bacteria can form thick biofilms [4, 5]. The bacterial density on the tongue surface has been shown to be related with the degree of oral malodor [6–8], indicating that in order to achieve long-lasting fresh breath the tongue bacterial density must be reduced and kept at a low growth rate to prevent fast recovery.

Numerous products are available for the control of oral malodor, generally categorized into chemical and mechanical methods. Chemical methods such as mouthrinses typically contain a combination of antimicrobial compounds (chlorhexidine or cetylpyridinium chloride) and metal ions (Zn^{2+}), which are very effective in reducing VSC [8–12]. However, mouthrinses are only effective for up to 3 h in general [13], due mostly to the masking of VSC, but have little effect on the tongue bacterial density [8]. Malodor levels increase

rapidly after a single usage [13], suggesting that those treatments cannot deliver a full day of fresh breath. Mechanical treatments such as tongue scrapers and brushes physically remove bacterial biofilms from the tongue surface. However, mechanical interventions only provide transient protection that can last for as little as 30 min [14]. This is presumably due to the limited biofilm removal from the tongue's complex surface. A higher reduction in malodor and bacterial density has been achieved by combining a mechanical with a chemical intervention [15], yet the overall evidence on the efficacy of chemical and mechanical interventions and their combination is still weak [16]. The sonic tongue brush investigated in this study was designed with soft silicone bristles to penetrate in between the tongue papillae and to provide a thorough mechanical biofilm removal. Bristle dimension and stiffness parameters were optimized based on analysis of human tongue papillae [4, 5, 17, 18]. The objective of this study was to test if a combined approach using a newly designed sonic tongue brush in combination with an antimicrobial spray could decrease oral malodor over a prolonged period of at least 6 h.

The hypothesis was to evaluate the effect of the treatments on oral malodor based on the organoleptic score and tongue bacterial density. An organoleptic score is determined by a trained odor judge who measures the strength of target odors and expresses the value in terms of a pre-defined scale [19]. It is the most widely accepted standard method to assess oral malodor and is a prerequisite for the diagnosis of halitosis in an individual [20, 21], as it represents what humans perceive. We also evaluated the efficacy of the treatment arms on the tongue bacterial density as this is a likely primary source of oral malodor [6–8].

This study represented the first clinical investigation with the TongueCare+ (TC) sonic tongue brush.

Materials and methods

Study design and participants

This randomized clinical investigation was carried out on a population with noticeable levels of oral malodor (organoleptic score, OS, higher than 2) at the University of West England (UWE) where all treatments and data collection took place in July–August 2014. The eligibility criteria included informed consent and availability at the specified study intervals and sampling times, a baseline organoleptic malodor score of >2, and at least 20 remaining permanent teeth and good oral hygiene and dental health. Exclusion criteria included: medical history of infectious diseases; severe caries, gingivitis or periodontitis; antibiotic medication within 1 month prior to the start of the trial or during the trial period; consumption of medicated sweets containing antimicrobial agents. Also subjects with diabetes mellitus, bronchitis, tonsillitis, sinusitis or other conditions that may contribute to oral malodor were excluded. All subjects were non-smokers.

Participants continued with their normal oral hygiene until the evening before the test, but were asked to abstain from it, and from food intake, in the morning of the test day. Consumption of foods associated with oral malodor (such as garlic, spices or alcohol) on the day prior to, and on the sampling day and using strongly perfumed cosmetics on the sampling day was not allowed. Water was allowed immediately after the 1 h and food after the 3 h following treatment.

The execution and design of this clinical investigation followed the guidelines proposed by Seemann and colleagues for oral malodor studies [21].

Thirty participants were screened to take part in this investigation, out of which 25 were selected by the principal investigator. They received four treatments one week apart in a crossover design. The randomisation consisted of issuing each person a different sequence of treatments, such that each person receives all treatments. The number of different possible sequences for four treatments is 16, which were randomised and allocated to the first 16 people, and the cycle of allocation repeated for the next nine participants. The 25 volunteers who were eligible to enter the trial were randomly assigned to the labels 1–25 using Excel software, and the treatment sequences were allocated against the trial numbers for each person. A co-investigator generated the allocation sequence, and an independent researcher assigned the treatments to participants accordingly.

Four participants failed the inclusion criteria on the test day and where therefore excluded. The group of 21 volunteers that participated in the study comprised of 11 male and 10 females age between 22 and 56 years (mean age male $=$ 38.3 and mean age female $=$ 37.0).

Ethical approval

The protocol for this clinical trial was approved by Philips internal ethical committee, the East of England Cambridge Central NRES committee (REC reference: 14/EE/0206) and UWE Ethics committee. The study was conducted in a manner consistent with the ethics of the 'Declaration of Helsinki'. All participants gave written consent before taking part in the study. The clinical investigation was registered at the ISRCTN registry (study identification number ISRCTN38199132) and the CONSORT statement checklist was followed for reporting.

Clinical procedures

Each participant was given an individual protocol, a diary and appointment dates and times for attending the laboratory. Each participant was coded, and a randomized treatment sequence was assigned to each. The experimental treatments consisted of: (1) TongueCare+ (TC, Sonicare, Philips), which consists of a pad with more than 200 microbristles mounted on an EasyClean Philips Sonicare handle. TC was used in combination with the antibacterial spray BreathRx (BRx, Sonicare, Philips). The active ingredients of BreathRx are 0.09% w/v cetylpyridinium chloride

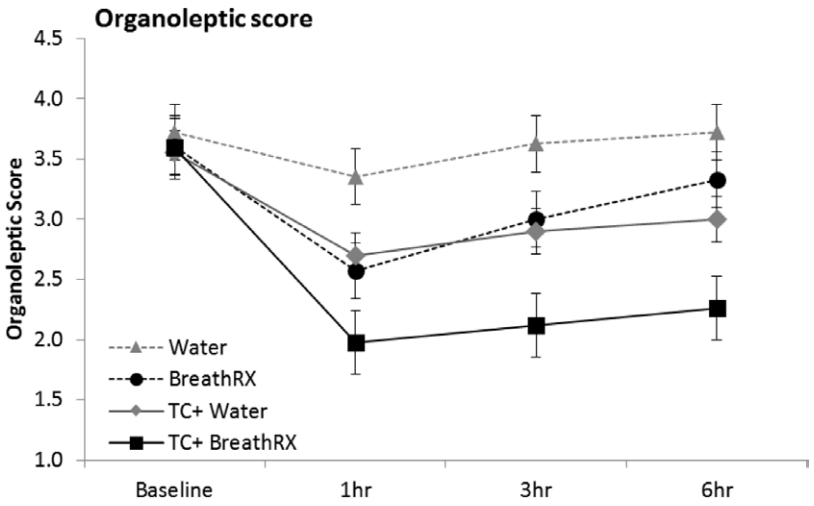

Figure 1. Average and 95% CI of organoleptic score at baseline, 1 h, 3 h and 6 h after each treatment.

(CPC) and 0.7% zinc gluconate. Non-active ingredients are: aqua, propylene glycol, PEG-40 hydrogenated castor oil, mint flavor, PEG 8 dimethicone, eucalyptus oil, thymol, sodium saccharin and acid blue 9.

Other treatments consisted of (2) TC used with water, (3) BRx spray alone, and (4) water alone. TC was used for a total of 60 s, in 20 s intervals. At each interval, three sprays of either BRx or water (approximately 0.6 ml) were placed on the tongue, followed by brushing. BRx or water alone treatments consisted of the same number and repetitions and volumes of the given spray alone.

On the day of their appointment, participants received their allocated treatment (1–4) and were instructed how to use it. Oral malodor and bacterial counts were assessed before treatment, and after 1 h, 3 h and 6 h post-treatment.

Organoleptic assessment

Oral malodor was assessed by organoleptic scoring. One trained odor judge scored breath odor levels using the 0–5 organoleptic scale outlined by [22] and modified in term of odor descriptive by [23]: 0 = no odor, 1 = barely noticeable, 2 = slight odor, 3 = moderate odor, 4 = strong odor, 5 = very strong odor The organoleptic judge was blinded to which treatment the participants had received.

Tongue bacterial density

Tongue scrape samples were taken using a sterile soft toothbrush with a 1 cm^2 flat bristle field [7] applied with gentle oscillations on the dorsum of the tongue, 7 cm from the tip. The sample was resuspended in 10 ml of phosphate buffered saline, serially diluted and plated with a spiral plater (Don Whitley, West Yorkshire, UK). Dilutions were plated on fastidious anaerobe agar (FAA; LabM, Bury, UK) supplemented with 7% defibrinated horse blood (TCS Biologicals, Bucks, UK) for isolation of anaerobes, and supplemented with vancomycin (2.5 mg L^{-1}) for isolation of strict gram-negative anaerobes. Plates were incubated anaerobically (Don Whitley, West Yorkshire, UK) with 10% CO_2, 10% H_2 and 80% N_2 at 37 °C degrees for 10 d. Samples were collected and processed by a blinded investigator who did not know the participant or treatment.

Statistical analysis

The primary hypothesis of this clinical investigation was that the TC + BRx delivers a significantly higher reduction on oral malodor at 6 h compared to all alternative treatments, assessed by the decrease both in organoleptic score (Delta OS) as the primary outcome and in bacterial tongue density as the secondary outcome. The sample size was calculated using a previous study [13]. A sample size of 21 volunteers sufficed to detect an OS difference of 0.8 with 95% power. The significance level used was 5%; the three main comparisons received a Bonferroni correction for multiplicity ($\alpha^* = \alpha/3 = 0.017$). A parametric and non-parametric tests were used (sign test and a *t*-test). For all the other time points, an ANOVA test was applied taking into account the four treatments and subjects ('repeated measures ANOVA'). The treatments were compared as pairwise comparisons using a multiplicity correction (Tukey).

Results

A total of 21 participants completed the study and received the four treatments, no dropouts occurred during the study. On average, the baseline OS for the 21 participants at the four appointments was 3.6 ± 0.2 (average ±95% CI) with a minimum of 3 and maximum of 4.5 corresponding to moderate to strong malodor. A single use of the TC + BRx significantly decreased oral malodor (p-value <0.05) to a score of 2.0 ± 0.2 (slight odor) at 1 h after treatment (figure 1). At 6 h the organoleptic scores increased by 0.3 to 2.3 ± 0.3 which was significantly lower than baseline ($p < 0.05$). A single usage of TC + water showed a

Table 1. Statistical results of organoleptic score and tongue bacterial density pairwise comparisons of the TC + BRx against all other treatment arms.

	One side *p*-value					
	Organoleptic score		Total anaerobes		Gram-negative strict anaerobes	
Hypothesis	*NP*	*P*	*NP*	*P*	*NP*	*P*
TC + BRx > BRx	$<10^{-6}$	1.6×10^{-8}	$<10^{-6}$	3.8×10^{-8}	2.1×10^{-4}	5.1×10^{-6}
TC + BRx > TC + Water	0.006	0.00003	1.3×10^{-6}	4.8×10^{-5}	1.3×10^{-3}	1.6×10^{-3}
TC + BRx > Water	$<10^{-6}$	3.1×10^{-6}	$<10^{-6}$	3.2×10^{-12}	$<10^{-6}$	4.6×10^{-10}

Note: *p*-values after Bonferoni correction. NP: non-parametric sign test. P: parametric *t*-test.

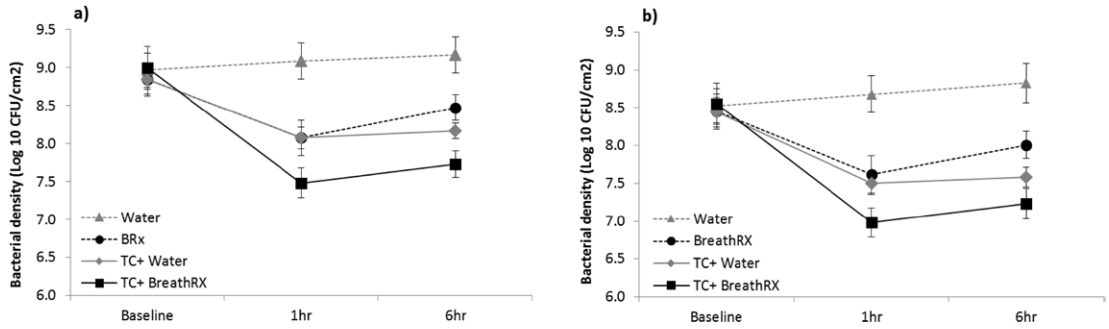

Figure 2. Average and 95% CI of (a) total anaerobes and (b) gram-negative strict anaerobes at baseline, 1 h, and 6 h after each treatment.

significant but moderate reduction in OS at 1 h, down to an average of 2.7 ± 0.2. At 6 h TC + water delivered a significant reduction from baseline, albeit minor (OS 3.0 ± 0.1). BRx showed a significant reduction in OS at 1 h, down to an average of 2.6 ± 0.2, but no significant difference from baseline was detected at 6 h (OS 3.3 ± 0.2). Rinsing with water was used as the negative control arm, which showed no change in the organoleptic score after treatment.

Comparing all treatment arms, TC + BRx had a significantly higher reduction in organoleptic score (Delta OS) score at each time point than the alternative treatments (*p*-value <0.017, after Bonferroni correction, table 1). The next most effective treatments in terms of Delta OS were BRx and TC + water which showed no significant difference between them, but both were significantly more effective than the control arm.

Bacterial density on the tongue surface, both total and strict gram-negative anaerobes, followed a very similar pattern as the organoleptic score (figure 2). The abundance of total anaerobes at baseline was on average 8.9 ± 0.5 Log_{10} CFU cm^{-2} (considering all treatment arms). At baseline, the strict gram-negative anaerobes were on average 8.5 ± 0.5 Log_{10} CFU cm^{-2}, representing 38% of the total anaerobes. TC + BRx significantly reduced the total anaerobes by 1.4 Log_{10} CFU cm^{-2} at 1 h after treatment and by 1.2 Log_{10} CFU cm^{-2} at 6 h, resulting in a bacterial density of 7.5 ± 0.2 and 7.7 ± 0.2 Log_{10} CFU cm^{-2}, respectively. The gram-negative strict anaerobes were also significantly reduced by 1.5 Log_{10} CFU cm^{-2} at 1 h and 1.3 Log_{10} CFU cm^{-2} at 6 h, resulting in 7.0 ± 0.2 and 7.2 ± 0.3 Log_{10} CFU cm^{-2} respectively. The TC + water treatment and the BRx alone treatment showed a significant decrease, but at a lower magnitude (0.68 and 0.37 Log_{10} CFU cm^{-2} reduction at 6 h, respectively) than TC + BRx, while a slight increase in the water control arm was detected. Pairwise comparisons of the treatment arms indicated that TC + BRx had a significantly greater reduction in the total and gram-negative strict anaerobes at 1 h and 6 h than all other alternative treatments (*p*-value <0.017, after Bonferoni correction, table 1). The next most effective treatments were BRx alone and TC + water. Both of these latter treatments showed no significant difference between them but were significantly more effective than the control arm at all time points.

We further investigated if there was a relationship between the tongue bacterial load and OS. A Spearman rank correlation test indicated a significant positive correlation between OS and total anaerobes bacterial density ($r^2 = 0.7, p < 0.01$) and with the gram-negative strict anaerobes ($r^2 = 0.6, p < 0.01$) (figure 3).

Overall, these data indicate that TC + BRx provides a noticeably clean breath at 6 h with an average improvement of 1.5 in the organoleptic score and a 1.3 Log_{10} CFU cm^{-2} reduction in the tongue bacterial density. Thus, on average volunteers went from having strong to slight malodor which they maintained for at least 6 h.

Discussion

In the present clinical investigation we evaluated the efficacy of a combined mechanical and chemical

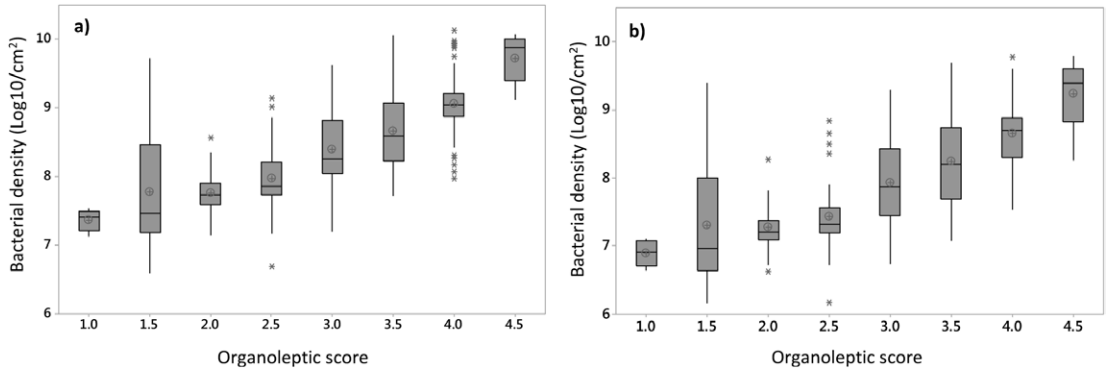

Figure 3. Comparison between organoleptic score and (a) total anaerobes ($r^2 = 0.7$, p-value <0.01) and (b) gram-negative strict anaerobes ($r^2 = 0.6$, p-value <0.01) in all pair data retrieved in this study (252 measurements). Box plot shows upper whisker representing the upper 25% of the distribution (excluding outliers), box representing the interquartile range middle 50% of the data. The middle line is the median and the circle is the mean. The lower whisker represents the lower 25% of the distribution (excluding outliers). *represents outliers which are observation that is beyond the upper or lower whisker.

intervention to reduce oral malodor. The primary objective was to determine if this combined approach could deliver a greater reduction in oral malodor and in bacterial density compared to the individual treatments. The newly designed sonic tongue brush (TC) was used in combination with either water or an antibacterial tongue spray (BRx) and was compared with the spray or water alone in a randomized crossover trial with a panel of 21 volunteers with one week washout period. We showed that a single use of the combined treatment reduced the bacterial load on the tongue surface and kept oral malodor at a low level for at least 6 h.

Oral malodor is primarily caused by the degradation of organic substrates by anaerobic bacteria in the oral cavity, mostly located on the tongue dorsum. Therefore, the treatment commonly recommended for oral malodor management is to mechanically decrease the bacterial density and their substrates, such as tissue and food debris, from the tongue [16, 24]. However, there is still insufficient evidence to indicate that the use of tongue scrapers or toothbrushes alone sufficiently reduces the tongue bacterial load [16, 25, 26]. The tongue is a flexible tissue with complex papillae and surface structures, such as pits, fissures and crypts where bacterial biofilms form and thrive [4, 5]. The use of mechanical methods such as tongue scrapers could flatten the papillae and likely remove only the top layer of the tongue coating and trap most of the in-between papillae biofilm underneath: therefore, the reduction in oral malodor may last for a very short time span [14, 27]. The tongue brush tested in this study was designed specifically to penetrate in between the tongue papillae, as bristle dimension and stiffness parameters were optimized based on analysis of human tongue papillae [4, 5, 17, 18]. Using the TC brush in combination with sonic motion was shown to remove a significant amount of the bacterial load, both total and strict gram-negative anaerobes (figure 2). This consequently leads to a reduction in oral malodor (figure 2) with a longer lasting effect than using tongue scrapers or toothbrushes [14, 27].

Mouthwashes containing antibacterial compounds, such as CPC and CHX, and zinc ions, are widely used for the treatment of oral malodor. Zn can reduce VSC by binding with compounds such as H_2S and CH_3SH and forming insoluble complexes (ZnS) which are not volatile and therefore non-odiferous [28–30]. Whilst mouthwashes have the potential to be very effective due to their antibacterial and oral malodor masking properties, they rarely provide long lasting relief. One possible explanation is that bacterial biofilms that produce the volatile gases involved in breath odor are mostly located deep inside the tongue papillae, where hydrodynamic mixing and diffusion of active ingredients is likely to be difficult due to the small papillae spaces, the viscosity of salivary molecules, and the low permeability of biofilms. In the present study the treatment with a rinse of spray (BRx) alone reduced the organoleptic score and bacterial density load significantly at all-time points in comparison with control, which is in agreement with previous studies [13, 31]. However, at 6 h the effect was small with the organoleptic score and bacterial density almost back to baseline level (figures 1 and 2).

The combination of mechanical (TC) and chemical (BRx) treatments delivered a significantly superior reduction in oral malodor compared to the individual treatments (table 1). This could be the result of both removing greater amounts of biofilm and delivering antimicrobial agents deep into tongue papillae structures. The enhanced reduction of bacterial density and retardation of bacterial re-growth should result in surviving microorganisms taking longer to recover to the pre-treatment density and corresponding malodor levels.

The results from this clinical investigation indicated that both the organoleptic score and the bacterial density can be significantly reduced with the treatment proposed, and that the tongue bacterial abundance is likely the key driver of intra oral malodor (figure 3). The abundance of total and strict gram-negative anaerobes at the start of the treatment were characteristic of a high odor group, while 6 h after treatment they were within

the range for low odor [7, 12]. The strict gram-negative anaerobes constituted a high proportion of total bacterial numbers (35 ± 5% of the total throughout the study), as is characteristic of high oral malodor [7, 32]. Within this broad group, there are several organisms that have been associated with oral malodor, such as *Prevotella*, *Leptotrichia,* or *Veillonella* [32–35]. However, some gram positives have also been strongly affiliated with oral malodor such as *Solobacterium moorei* [36], and it is still unclear what the extent of bacterial diversity contribution is to oral malodor. In the present study we did not further distinguish other microorganisms that could have been selectively affected by the treatment; however a single treatment is unlikely to have an impact on bacterial ecological composition.

In the present clinical investigation we demonstrated that a single usage of a combined mechanical and chemical therapeutic approach, TongueCare+ with BreathRx tongue spray, can significantly decrease the tongue bacterial biofilm load. Subsequently, this leads to a reduction in oral malodor for up to 6 h, which is a significant improvement over each treatment separately, providing a more effective and long-lasting treatment option for people suffering from oral malodor.

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Conflict of interest statement

The authors declare that there are no conflicts of interest in this study. This study was funded by Philips Research and Philips Oral Healthcare. Three of the authors are employees of Philips Research. No external funding, apart from the support of the authors' institution, was available for this study. In this work Sonicare power toothbrushes, brush heads, and tongue spray were used which are owned by Philips.

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