Plaque control and halitosis management using a two-phase mouthwash. A study on healthy volunteers

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Introduction
Oral hygiene plays a fundamental role in preventing common oral pathologies, including caries and periodontal disease, which may lead to tooth loss if left untreated (1). Oral hygiene mainly involves removing plaque, the bacterial biofilm that forms over teeth. Plaque-forming bacteria are a subset of the oral microbiome, which includes species belonging to approximately 700 taxa (2). Supragingival plaque control is of paramount importance in preventing oral diseases and tooth loss (3) especially consequent to periodontitis, which is correlated to a high prevalence of microbial pathogens including members of the red complex species (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia) and orange complex species (Fusobacterium nucleatum and Prevotella intermedia, Campylobacter rectus), as defined by Socransky et al. (4). Standard oral hygiene practices include periodic mechanical plaque removal by means of a toothbrush, in conjunction with...
with a toothpaste, and using dental floss. However, motivation and manual dexterity may limit their effectiveness (5,6).

Species of the oral microbiota may also be responsible for less serious, but maybe more life-quality disruptive disorders, like halitosis, whose prevalence in adolescents and adults peaks at about 32% (7). In general, halitosis is mainly caused by Gram-negative anaerobic bacteria (8) of the biofilm covering the teeth, as well as the tongue (9). The products of bacterial metabolism involved in halitosis include mainly volatile sulphur compounds (VSCs), as well as indoles, skatoles, amines, and ammonia. VSCs are mainly hydrogen sulphide (H₂S), methyl mercaptan (CH₃SH), and dimethyl sulphur (CH₃)₂S, which are thought to be the main cause of oral malodour (10). Bacteria-producing VSCs include again Gram-negative Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia also linked to periodontitis as well as Fusobacterium nucleatum, the commensal bacteria Actinomyces odontolyticus (Gram-positive), Veillonella dispar (Gram-negative), and Solobacterium moorei (Gram-positive) (11-14). These bacteria, most of which also cause periodontal disease, degrade proteins of exfoliated epithelial cells, blood cells, food, and other debris to cysteine and methionine, which degrade further to form VSCs (10). Gram-positive bacteria are weak producers of VSCs per se but can deglycosylate some proteins facilitating their degradation by Gram-negative ones (15).

Mouthwash formulations have been proposed as a useful adjunct to tooth brushing and the use of dental floss, to improve plaque control and manage halitosis (16,17). Mouthwashes are also easy to use for people lacking dexterity, and as they spread throughout the mouth and so they act on all oral tissues (17,18). Cetylpyridinium chloride (CPC) is an anti-microbial cationic surfactant that acts by disrupting the bacterial cell membrane, leading to the leakage of intracellular material and finally to cell death (19). Its possible anti-plaque activity was first suggested by Schroeder et al. in 1962 (20) and confirmed by first clinical trials in the late 60’s and early 70’s on 0.025-0.05% w/v CPC mouthwashes. These were found to reduce the plaque index (PI) (21) by between 15% and 30% when used for a period of between three weeks and six months (22-24). Essential oils (EOs) are another component of clinically studied and marketed mouthwashes due to their anti-halitosis effect (25-27) and safety (28). Mouthwashes containing EOs have been shown to allow better plaque control than placebo solutions, yet meta-analyses report high heterogeneity in results, due to both the variations in design of the trials being assessed, and because of the variety of EOs used in mouthwash formulations (29,30). Accordingly, one should assume that different EO-based formulations display different safety and effectiveness profiles, until proven otherwise. Results of recent systematic reviews and meta-analyses also suggest that mouthwashes combining CPC and EO might be more effective than those that are EO- or CPC-based only (30,31). Further, two-phase (oil/water) mouthwashes containing quaternary ammonium salts (like CPC) have long been suggested to be effective in binding and desorbing oral microorganisms, as these cationic species can increase adhesion of microorganisms and oral debris to oil droplets, thus facilitating their removal (32-34). Dentyl mouthwash (Venture Life Group PLC, Bracknell, UK) is an innovative two-phase (oil/water based) oral rinse containing Cetylpyridinium Chloride (CPC), Sodium Fluoride, Xylitol, flavourings and essential oil as the main ingredients, and it claims to effectively remove plaque and manage halitosis. The aim of this study is to quantitively assess, under controlled conditions, to what extent it reduces plaque and enables halitosis management.

Materials and methods
This clinical study involved 20 healthy volunteers suffering from plaque and halitosis. The study was carried out at a single centre in Italy (Eurofins Biopharma, Rome), from June 11, 2019 to July 16, 2019 in accordance with the principles outlined in the Helsinki Declaration (1964), the ICH E6 (R1) Good Clinical Practice, the European 2001/20/EC Directive for conducting clinical trials on drugs and the recommendations of Colipa - The European Cosmetic and Perfumery Association (2008) guidelines for the evaluation of efficacy of cosmetic products. The study was performed by a competent investigator (dentist) and trained and qualified technical staff.

Subjects, of both genders, who provided their informed consent, were included if aged between 18 and 65 and suffering from plaque or halitosis. Exclusion criteria were: subjects suffering from any oral/gingival diseases; planned to undergo, during the study period, any kind of dental care or any pharmacological treatment which may have interfered with the interpretation of the study result; using any dental prosthesis; pregnant or breastfeeding. Subjects were also excluded if assessed to be potentially non-compliant with the study protocol and if concomitantly participating in any other study.

Investigational product
The mouthwash under investigation (Venture Life Group PLC, Bracknell, UK) is an oral rinse containing Cetylpyridinium Chloride (CPC), Sodium Fluoride, Xylitol, flavorings and essential oils as the main
ingredients. While the bottle is left undisturbed, the two brightly colored oil- and water-based phases remain separate. Before using the mouthwash, the user is instructed to shake the bottle until a homogeneous suspension is achieved; thorough mixing is easily detected as the suspension will have a uniform color throughout, midway between the colors of the two separate phases. The user then fills the bottle cap (15ml), sips the mouthwash - just into his/her mouth - and rinses the oral cavity for 30 seconds. The mouthwash is then spit out and not swallowed. The users are instructed to use the product twice a day, after brushing the teeth, and to not eat or drink for 30 minutes after rinsing.

Study plan
All participants were provided with the mouthwash under investigation and a standard toothpaste without menthol. The short-term observation period lasted five days. Subjects were recruited on the first day and were instructed to clean their teeth in the evening with the standard toothpaste and rinse their mouth with 15ml tap water. The following morning the subjects’ Plaque Index (PI), Full Mouth Plaque Score (FMPS), Gingival Index (GI) and Volatile Sulphur Compounds (VSCs) were evaluated and the main periodontopathogenic bacteria, belonging to the species of interest (see following paragraphs for details) were quantified. This point in time, namely 12 hours after brushing with the standard toothpaste and rinsing with water, was regarded as the baseline starting point (Time zero, T0) to which all subsequent measurements were compared. The volunteers were then instructed to continue their usual oral hygiene routine for three days and, on the evening of the fourth day, to brush their teeth with the standard toothpaste and follow with a 30 second rinse with 15ml experimental mouthwash. The next morning, specifically 12 hours after using the mouthwash (T12h), the subjects had their PI, FMPS, GI and VSC assessed, as well as periodontopathogenic bacteria of the species of interest quantified. They then underwent a full clinical oral examination and rinsed their mouth with 15ml experimental mouthwash for 30 seconds. One hour after rinsing with the experimental mouthwash (T1h), their VSC and main periodontopathogenic bacteria were quantified again.

The long-term observation period involved the subjects brushing their teeth with the standard toothpaste, and rinsing with 15ml experimental mouthwash for 30 seconds, twice a day (morning and evening) for the following 28 days. They also kept a daily log of any discomfort or unpleasant reaction they might have felt. The morning after completing this 28-day period, the volunteers had their PI, FMPS and GI measured once again, and underwent a thorough clinical mouth assessment. The daily log was examined by the investigator and the subject questioned if necessary. Subjects were also asked to fill in a questionnaire to provide their final subjective judgement on the mouthwash (see following paragraph).

Endpoints of interest and their measurement
The Full Mouth Plaque Score (FMPS) (35) was measured as the percentage of teeth presenting plaque. The Plaque Index (PI) (21,36) and the Gingival Index (GI) (36,37) were assessed during the clinical examination by scoring the plaque amount and the gingival inflammation status as reported in Table 1. Periodontal bacterial identification and quantification of *Aggregatibacter actinomycetem comitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* was carried out using a commercial identification kit (PeriodontalDNA test kit, Curasept). In short, the supragingival plaque was first removed from the collection site and dried using sterile cotton. A sterile paper cone was then inserted into the gingival sulcus for about 30 seconds, then transferred to a...
sterile tube. This collection procedure was repeated at two additional sites, storing all cones in the same tube. After labelling, the tube was sent to the analysis lab. Here, the bacterial DNA was extracted from the cones and analyzed by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR), using appropriate primers to amplify DNA of the bacterial species of interest, after building appropriate calibration curves.

Volatile Sulphur Compounds were measured by gas chromatography using an oral chromatography device (Oral Chroma CHM2, GVZ components, Rho, Italy). A syringe was inserted into the subject’s oral cavity until the flange reached the lips, and they were asked to bite gently on the syringe to block it. The subject was then asked to close his/her mouth tightly and breathe through the nose, keeping the oral cavity unventilated for at least 30 seconds, and not touch the syringe tip with their tongue. The syringe piston was then retracted to fill the syringe with a breath sample from the oral cavity. A 1cc sample was injected into the chromatograph and VSCs were measured.

The subjective questionnaire the subjects were asked to fill in at the end of the long-term observation period required them to indicate on a 0 to 3 scale (0, disagree; 1 slightly disagree; 2, slightly agree; 3, agree) his/her own level of agreement to nine statements concerning the mouthwash under investigation (Table 2) regarding its safety, tolerability, pleasantness and performance.

The daily log was assessed as follows: the investigator recorded any visible clinical sign or feeling of discomfort, assessing its intensity on a three-category scale (slight, moderate, severe). For both, the investigator noted the location, duration, time of occurrence after using the mouthwash, frequency, course, and medical treatment possibly undertaken. This was later used to calculate the fraction of subjects who were reactive to the mouthwash. The percentage of subjects exhibiting clinical signs or sensations of discomfort attributable to the mouthwash was then used to assess its oral compatibility according to Table 3.

### Statistical analysis
Normality of quantitative variables was checked using the Shapiro-Wilk test, with a significance level \( \alpha=0.01 \). Quantitative variables were summarized by using their frequency, arithmetic mean and standard deviation. Categorical variables were summarized by using frequency distributions and percentages. Values of endpoints at different experimental times (T12h, T1h, T28d) were compared to baseline (T0), by means of t-tests for paired data if variables were found to be normal, or using Wilcoxon signed-rank tests for paired data if they were not. Results of statistical tests were regarded as significant if \( p<0.05 \). Statistical analyses were carried out using standard statistical software (R, R Foundation for Statistical Computing (2019) (38) and Origin 2020, OriginLab Corporation, MA, USA).

<table>
<thead>
<tr>
<th>Oral compatibility</th>
<th>% of subjects exhibiting clinical signs imputable to the mouthwash</th>
<th>% of subjects exhibiting sensations of discomfort imputable to the mouthwash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Good</td>
<td>0%</td>
<td>&lt; 30%</td>
</tr>
<tr>
<td>Moderate</td>
<td>&lt; 20%</td>
<td>whatever</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>30 to 50%</td>
</tr>
<tr>
<td>Bad</td>
<td>20%</td>
<td>whatever</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>&gt; 50%</td>
</tr>
</tbody>
</table>

Table 3 Scores allowing the investigator to assess the mouthwash oral compatibility
Results

Twenty healthy volunteers, aged from 18 to 64 years (average, 50.9 ± 11.0) participated in the study. Thirteen were women (65%) and seven were men (35%). Thirteen did not take any medication; seven were taking medication that was not deemed to interfere with the study results (three subjects were taking medication for hyperthyroidism, two were taking medication for diabetes; three for hypertension; and one person was taking medication for depression and epilepsy, with each subject potentially taking medications for more than one disorder). All subjects completed the study. Subjects did not report any sign of discomfort, and no adverse clinical signs related to the mouthwash were observed throughout the whole study observation period. Accordingly, the mouthwash was considered to have a very good oral compatibility.

Short-term assessment

Twelve hours after brushing with the standard toothpaste and rinsing with the mouthwash (T12h), and compared to T0, the plaque index (PI) had decreased by 35.2%, the gingival index (GI) had decreased by 36.6% and the full mouth plaque score (FMPS) by 24.7%, all changes being statistically significant (p<0.05) (Table 4, Figure 1).

Table 4 Short- and long-term results concerning the plaque index (PI), the gingival index (GI) and the full mouth plaque score (FMPS). Twelve hours after brushing with the standard toothpaste and rinsing with the mouthwash (T12h), compared to brushing with the toothpaste and rinsing with water (T0), the plaque index (PI) decreased by 35.2%, the gingival index (GI) decreased by 36.6% and the full mouth plaque score (FMPS) by 24.7. After 28 days brushing with the standard toothpaste and rinsing with the experimental mouthwash twice a day, all oral health status indices had further significantly decreased compared to T0. (*) marks statistical significance (p<0.05). W, Wilcoxon Signed-Rank test for paired data; T, Student t-test for paired data. NS, not significant

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>T0 (12h after the brushing with the standard toothpaste and rinsing with water)</th>
<th>T12h (12h after brushing with the standard toothpaste and rinsing with the mouthwash)</th>
<th>T28d (After brushing teeth and rinsing twice a day with the mouthwash)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque Index (PI)</td>
<td>1.8 ± 0.6</td>
<td>1.2 ± 0.6 (-35.2%) *&lt;sub&gt;T&lt;/sub&gt;</td>
<td>0.7 ± 0.4 (-61.2%) *&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
<tr>
<td>Gingival Index (GI)</td>
<td>1.5 ± 0.7</td>
<td>1.0 ± 0.6 (-36.6%) *&lt;sub&gt;W&lt;/sub&gt;</td>
<td>0.6 ± 0.4 (-63.3%) *&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
<tr>
<td>Full Mouth Plaque Score (FMPS)</td>
<td>62.5 ± 0.3%</td>
<td>47.1 ± 0.2% (-24.7%) *&lt;sub&gt;W&lt;/sub&gt;</td>
<td>33.4 ± 0.2% (-46.5%) *&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Results concerning periodontopathogenic bacteria and VSC variation over the short-term observation periods are reported in Figures 2 and 3 and Table 5. Twelve hours after brushing with the standard toothpaste and rinsing with the experimental mouthwash (T12h), all periodontopathogenic bacterial species under consideration except *Tannerella forsythia* had decreased more than 80% compared to T0, the difference being statistically significant (p<0.05) (Figure 2). Twelve hours after brushing with the standard toothpaste and rinsing with the experimental mouthwash (T12h) mouthwash VSCs were reduced by 35.5% compared to T0, i.e. compared to 12 hours after brushing with the standard toothpaste and rinsing with water. One hour after rinsing with the mouthwash again (T1h), VSCs were reduced by 62.9% compared to T0. Both changes were statistically significant (p<0.05) (Figure 3).

Long-term (28 days) assessment

At the end of the 28 day usage period, the oral health status indices had undergone a further, statistically significant, reduction (p<0.05) compared to T0: the plaque index (PI) had decreased by 61.2%, the gingival index (GI) by 63.3% and the full mouth plaque score (FMPS) by 46.5% (Figure 3). Most subjects (> 85%) favorably evaluated the mouthwash with regard to both its tolerability and its performance (Table 6).

Discussion

The results of the present study show that the mouthwash under investigation has an optimal safety profile, as the investigators observed no adverse clinical signs that could be attributed to its use, and none of the subjects reported any discomfort. This finding was confirmed by the results of the subjective questionnaire administered to subjects at the end of the 28 day period, with 100% reporting optimal tolerability and the absence of any irritation. Even stinging and dryness were scarcely felt by the subjects (95% reported no stinging sensation, and 100% no dryness). With regard to performance, and

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**Table 4** Short- and long-term results concerning the plaque index (PI), the gingival index (GI) and the full mouth plaque score (FMPS). Twelve hours after brushing with the standard toothpaste and rinsing with the mouthwash (T12h), compared to brushing with the toothpaste and rinsing with water (T0), the plaque index (PI) decreased by 35.2%, the gingival index (GI) decreased by 36.6% and the full mouth plaque score (FMPS) by 24.7. After 28 days brushing with the standard toothpaste and rinsing with the experimental mouthwash twice a day, all oral health status indices had further significantly decreased compared to T0. (*) marks statistical significance (p<0.05). W, Wilcoxon Signed-Rank test for paired data; T, Student t-test for paired data. NS, not significant.
Figure 1 Reduction of PI, FMPS and GI over time. T0 (baseline), after brushing with the standard toothpaste and rinsing with water. T12h, 12 hours after brushing with the standard toothpaste and rinsing with the experimental mouthwash; T28d, at the end of the 28 days observation period. (*) marks statistical significance. Comparisons with respect to baseline (T0) were carried out using t-tests for paired data because variables were found to be normal, except for GI and FMPS at T12h which were compared to T0 using Wilcoxon Signed-Rank tests for paired data.

Figure 2 Reduction in Aggregatibacter actinomycetem comitans, Tannerella forsythia, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola twelve hours after brushing teeth with the standard toothpaste and rinsing with water (T0, baseline); 12 hours after brushing teeth with the standard toothpaste and rinsing with the experimental mouthwash (T12h); one hour after rinsing.
as far as plaque and oral health are concerned, the mouthwash was found to be effective on both the short- and the long-term. The first use alone led to a significant decrease both of PI (-35.7%) and FMPS (-24.7%) compared to baseline; these parameters further improved over the 28 days period (-61.2% and -46.5% respectively), indicating that continuous use of the mouthwash facilitates the effective reduction and management of plaque. Such short- and long-term plaque control effects led to a significant, and clinically relevant, improvement in the subjects’ oral health status, as measured by a significant reduction in the GI (-63.3% after 28 days). The results of the present study also show the mouthwash was effective in managing halitosis over a 12 hour period, as 12 hours after first use VSC levels were significantly lower (-35.5%) than before use. On a very short term basis, one hour after use, the anti-halitosis effect was even higher (-62.9%). These results are consistent with those concerning the bacterial population that was investigated: 12 hours after first use the reduction compared to baseline was variable, depending on the bacterial species under consideration, (the range being -8.7% to -67.9%, the decrease of Tannerella forsythia being not significant), while one hour after use the decrease for all bacterial species (including Tannerella forsythia), compared to baseline, was > 80%. Objective measurements are consistent with the subjective perception of the subjects, who reported the mouthwash freshened breath, and considered the effect to be long-lasting; good taste and good appearance were also reported. Significantly, the subjects also agreed that after rinsing, plaque/bacteria/debris residues could be seen in the sink (a result of the oil/water micelles embedding plaque, bacteria and food debris). These subjective, positive perceptions indicate that users of the experimental mouthwash may feel highly motivated to adhere to the practice of mouth rinsing, in part due to the long-lasting sensation of freshness, but also to the visual confirmation (seeing plaque/debris in the sink) that the mouthwash is actually effective, as proven by the significant PI and FMPS reduction detected in this study. Such factors, perhaps less relevant

![Graph](image1)

with The experimental mouthwash (T1h). (*) marks statistical significance. Comparisons with respect to baseline (T0) were carried out using Wilcoxon Signed-Rank tests for paired data because variables were found to be non-normal, except for the quantity of Tannerella forsythia at T12h and Treponema denticola at both T12h and T1h, which were compared to T0 using t-tests for paired data

![Graph](image2)

Figure 3 Reduction in VSCs twelve hours after brushing teeth with the standard toothpaste and rinsing with water (T0, baseline); 12 hours after brushing with the standard toothpaste and rinsing with The experimental mouthwash (T12h); one hour after rinsing with The experimental mouthwash (T1h). (*) marks statistical significance. All comparisons have been carried out using Wilcoxon Signed-Rank tests for paired data.
in educated/well-informed adults, might be more beneficial in helping less motivated subpopulations, such as adolescents (39-41) to acquire and maintain appropriate oral hygiene habits. The mouthwash under investigation is, in fact, well suited to this subpopulation. The results of the present study are consistent with those reported in the literature. In their systematic literature review concerning anti-plaque agents, Spivakovsky et al. (42) conclude that formulations that allow plaque control consistently provide statistically significant improvements in terms of gingival inflammation in patients with gingivitis, and in plaque indices. The effect of different mouthrinses on GI and PI reduction is reported to vary within a wide range (15-70%) in different systematic reviews and meta-analyses (29,43,44), the reasons for such variability being again a high heterogeneity of published studies regarding the subject characteristics at baseline, and the experimental design. The mouthwash investigated in the present study, therefore, provides clinical benefits that are consistent with those reported in literature, with the PI and GI score reduction being among the highest reported. This does not come as a surprise after the extensive and previous studies demonstrating that the 2-phase oil:water formulation of the experimental mouthwash, containing low concentrations CPC, efficiently binds and desorbs oral microorganisms (33). The results of the present study are also consistent with those reported in literature with regard to halitosis reduction: a Cochrane systematic review on the effect of mouthwashes on halitosis (45) reported the reduction of VSCs over a 2-week / 4-week period to range from 30% to 60%, depending on the mouthwash investigated; Blom et al. (46), while not providing an analysis regarding the extent of VSC reduction that may be achieved with mouthwashes over a short- or a long-term basis concluded, in their systematic literature review, that mouth rinses containing antimicrobial agents such as cetylpyridinium chloride may play an important role in reducing the levels of halitosis-producing bacteria on the tongue, and can be effective in neutralising odoriferous sulphur compounds. However, any comparison of the present study’s results with those reported in the literature, as well as among the several studies presently published on the subject, should be carried out with caution considering a lack of standardization of participants at baseline, as well as heterogeneity in measuring VSCs (45,47). The anti-plaque effect of the mouthwash under investigation was expected, given the known anti-microbial properties of CPC (19), and

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>T0 (12h after brushing with the standard toothpaste and rinsing with water)</th>
<th>T12h (12h after brushing with the standard toothpaste and rinsing with the mouthwash)</th>
<th>T1h (1 hr after rinsing with the mouthwash)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VSC</td>
<td>536.6 ± 557.6</td>
<td>346.1 ± 464.5 (-35.5%) *w</td>
<td>198.9 ± 256.3 (-62.9%) *w</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetem comitans (x10^6)</td>
<td>1.03 ± 1.33</td>
<td>0.88 ± 0.80 (-14.4%) *w</td>
<td>0.17 ± 0.14 (-83.6%) *w</td>
</tr>
<tr>
<td>Tannerella forsythia (x10^6)</td>
<td>0.17 ± 0.15</td>
<td>0.18 ± 0.21 (-8.72%) NS, T</td>
<td>0.02 ± 0.02 (-88.6%) *w</td>
</tr>
<tr>
<td>Porphyromonas gingivalis (x10^6)</td>
<td>0.60 ± 0.60</td>
<td>0.23 ± 0.19 (-61.3%) *w</td>
<td>0.08 ± 0.08 (-86.0%) *w</td>
</tr>
<tr>
<td>Prevotella intermedia (x10^6)</td>
<td>0.94 ± 0.88</td>
<td>0.30 ± 0.24 (-67.9%) *w</td>
<td>0.11 ± 0.06 (-88.0%) *w</td>
</tr>
<tr>
<td>Treponema denticola (x10^6)</td>
<td>6.52 ± 2.20</td>
<td>4.76 ± 2.89 (-27.0%) *t</td>
<td>1.00 ± 0.80 (-83.6%) *t</td>
</tr>
</tbody>
</table>

Table 5: VSCs and periodontopathogenic bacterial reduction after the short-term usage period. Twelve hours after brushing with the standard toothpaste and rinsing with the mouthwash (T12h), VSCs were reduced by 35.5% compared to T0 (twelve hours after brushing with the standard toothpaste and rinsing with water). On the same day, one hour after rinsing with the mouthwash only (T1h), VSCs were reduced by 62.9%. Twelve hours after rinsing with the mouthwash (T12h), Tannerella forsythia quantity did not vary, while all other species decreased significantly within a 27.0-67.9% range compared to T0. One hour after using the mouthwash the decrease of all periodontopathogenic bacterial species considered was greater than 80%, compared to T0. (*) marks statistical significance (p<0.05). W, Wilcoxon Signed-Rank test for paired data; T, Student t-test for paired data; NS, not significant.
the results of microbiological tests on the bacterial species which cause halitosis and periodontal diseases such as those investigated in the present study (48). The experimental mouthwash required continuous use to achieve a significant reduction in oral colonization by all species, indicating regular use is necessary for the mouthwash to effectively control this oral microbiome subpopulation. One confounding factor of the present study is that specific thresholds for plaque and halitosis were not set for recruitment; accordingly, participants showed a high degree of variability at baseline (the standard deviation for these parameters being about the same as the quantity of interest). However, the heterogeneity of the present study’s sample may be regarded as not dissimilar to that of the general population. As such, the results of this pilot investigation might better describe the average outcome that may be expected under real-life conditions. In the present study no stratification, or uni- or multi-variate correlation analyses could be carried out between the endpoints of interest and the characteristics of the subjects at baseline, as the sample size was deemed too small for such analyses to be meaningful. For this, future studies should be

<table>
<thead>
<tr>
<th>Items</th>
<th>Answers</th>
<th>Number of subjects</th>
<th>Percentage of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
</tr>
<tr>
<td>The mouthwash is well tolerated</td>
<td>17</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>The mouthwash does not irritate gums</td>
<td>16</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>The mouthwash makes the breath fresh</td>
<td>11</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>The mouthwash lifts plaque from your teeth which you can see in the sink</td>
<td>5</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>The mouthwash gives a long-lasting fresh sensation</td>
<td>9</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>The mouthwash has a good taste</td>
<td>11</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>The mouthwash has a good appearance</td>
<td>7</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>The mouthwash does not sting your mouth</td>
<td>16</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>The mouthwash does not dry your mouth</td>
<td>15</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6: Results of the subjective questionnaire administered at end of the study. Most subjects (≥ 85%) appreciated the mouthwash with regard to both its tolerability and its performance.
carried out on a greater number of subjects, possibly with the inclusion of a control or placebo group to better characterize the experimental mouthwash’s favorable plaque control and halitosis management properties highlighted in the present study. Further, the subject of specific, appropriately designed future comparative studies, should be also the efficacy of the experimental mouthwash in neutralizing odoriferous sulfur compounds compared to other marketed mouthwashes.

Conclusion
The two-phase mouthwash (Venture Life Group PLC, Bracknell, UK) containing Cetylpyridinium Chloride (CPC), Sodium Fluoride, Xylitol, flavorings and essential oils as main ingredients was effective in controlling plaque formation and reducing halitosis from its very first use, and its anti-halitosis effects were still significant 12 hours after rinsing. Its use was also associated with a significant decrease in periodontopathic bacteria. Using the mouthwash for four weeks led to a significant reduction of the plaque index, the full mouth plaque score, and the gingival index, significantly improving the oral health status of the subjects participating in the study. Given that it also caused no adverse effects, showed very good tolerability and was highly valued by the participants, Dentyl is regarded as a safe, effective and useful adjunct to everyday oral hygiene routine.

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Conflict of interest
Valentina Molteni, Eleonora Corti and Stefania Abbattista are employees of Biokosmes S.r.l., a Venture Life Group PLC, Bracknell, UK Company. Biokosmes S.r.l. did not interfere with study execution, results or collection, analyses and interpretation of data. This study was funded by Biokosmes S.r.l.

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