Effect of Inhabitation Mouthwash Solution Containing Chlorine Dioxide (Pro Fresh®) on Oral Malodor

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Received 23 December 2014; accepted 8 February 2015; published 15 February 2015

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Abstract

Recently, the interest in oral malodor has been grown and the number of people having trouble with oral malodor is increasing year by year. Generally, as the branch of medicine which deals with patients complaining about oral malodor, dentistry is mostly chosen. On the current situation, however, the dental office side typically finds it difficult to deal with several kinds of oral malodor which show no improvement even though cavities or periodontal disease is completely cured. Main components to cause oral malodor are volatile sulfur compounds (methyl mercaptan, dimethyl sulfide, hydrogen sulfide); in the present study, therefore, the effect of mouthwash solution containing chlorine dioxide to relieve oral malodor and increase saliva production was tested. The subjects were 92 patients (Control group: 2 males, 3 females, age; 30.8 ± 4 years old, Mouthwash group: 28 males, 59 females, age: 36.9 ± 1.3 years old) who visited the hospital complaining about oral malodor. Intraoral gas, exhaled gas, the saliva production at rest, the ability for salivation and the buffering ability of saliva were examined twice, before the subjects used the mouthwash solution every day for one month. The simple chromatography oral chroma, which was insulated from the influence of temperature and humidity and also was able to measure with a high degree of accuracy, was used to measure odor as the device analysis method. The control group did not change in VSCs, saliva production and the buffering ability of saliva. After using mouthwash solution, the concentration of the 3 major components gas of intraoral gas and exhaled gas, i.e. methyl mercaptan, dimethyl sulfide, hydrogen sulfide, were all decreased. As for hydrogen sulfide, intraoral gas (from 225.8 ± 28.1 ppb to 41.1 ± 8.8 ppb) and exhaled gas (from 212.0 ± 50.4 ppb to 34.6 ± 16.6 ppb) were significantly decreased.

http://dx.doi.org/10.4236/health.2015.72025
ppb) was significantly decreased. Also, saliva production at rest (from 1.3 ± 0.1 ml to 1.7 ± 0.1 ml) and the ability to salivate (from 4.5 ± 0.3 ml to 5.1 ± 0.3 ml) were both significantly increased. As for the buffering ability of saliva, significant changes were not detected on the change of salivary pH after using mouthwash solution, and it did not have any effects on the buffering ability of saliva. In conclusion, these results suggest that the mouthwash solution containing chlorine dioxide has significant effects on inhibiting malodor.

**Keywords**

Chlorine Dioxide, Oral Malodor, Volatile Sulfur Compounds, Intraoral Gas, Exhaled Gas

### 1. Introduction

Recently, because of the increase in the awareness for oral cleaning, there are an increasing number of people who have high interest in oral malodor and also have trouble with oral malodor.

Generally, as the branch of medicine which treats patients complaining about oral malodor, dentistry is mostly chosen. Under the current situation, however, the dental office side finds it difficult to deal with several kinds of oral malodor.

Oral malodor is divided into oral malodor (odor) and malodor syndrome (disease) by the guideline of the Japanese Academy of Malodor Syndrome. It is also defined as follows: oral malodor is the collective term of odor that the identical person or the third person feels uncomfortable with. Malodor syndrome is the symptom that the identical person feels anxiety about oral malodor because of physiological, organic (physical) and psychological reasons [1].

It is known that the components of oral malodor are associated with anaerobic bacteria which are the main cause of oral malodor [2]-[5], and volatile sulfur compounds (hereinafter referred to as VSCs) contain methyl mercaptan (CH₃SH), dimethyl sulfide ((CH₃)₂S) and hydrogen sulfide (H₂S) [3] [6].

VSCs is the major causal component of them all, and the major source of VSCs is the tongue coat. The occurrence of VSCs from the tongue involves anaerobic bacteria [3] [7]. Moreover, it is considered that there are 8,000,000 patients who suffer from dry mouth with the chief complaint of oral dryness and the patients who potentially have dry mouth can be estimated as 30,000,000 in Japan.

To prevent oral malodor and dry mouth, accelerating salivary secretion using the physiology of the oral cavity, doing oral cares at home, functional cleaning of teeth and mouth with professional cares at dental medical institutions, and using mouthwash solution are common. The mouthwash solution designed to prevent oral malodor has been developed, marketed and widely used.

As medicinal properties, chlorhexidine, triclosan, refined oil, dehydroascorbic acid, hydrogen peroxide, hydrogen dioxide and metal ion etc. are used for the mouthwash solution, and their effectiveness has been reported [8]-[10].

Chlorine dioxide is generated by the reaction of sodium chlorite and citric acid (or hydrochloric acid), or by the reaction of sodium chlorite, sodium hypochlorite and citric acid (or hydrochloric acid), and oxygen is generated at the time of degradation. As nascent oxygen is in condition of active oxygen and has powerful antiseptic property, chlorine dioxide is expected to be effective in preventing oral malodor by supplying oxygen to the anaerobic bacteria which produce the causative substances of oral malodor.

Moreover, chlorine dioxide dissolves VSCs which is well known as the causative substances of oral malodor [10] [11].

In the present study, therefore, the experiments were conducted to verify the effect of the marketed mouthwash solution (Pro Fresh®), which contained chlorine dioxide on the inhibition of oral malodor and dryness in oral cavity.

### 2. Material and Method

#### 2.1. Subjects

The subjects were the patients who visited hospital with complaints of oral malodor in this study. The subjects...
were 92 volunteers obtained consent by a purpose of this study and selected by the entry criterion (the interval of the first oral malodor test and the second malodor test was less than 1 month), the exclusion criterion (excluded patients with systemic illness, Sjogren’s syndrome which decreases saliva, smoking habits and severe periodontal disease) and the primary screening. In the control group, 5 people (2 males, 3 females, age; 30.8 ± 4.0 years old), mouthwash group assumed 87 people (28 males, 59 females, age: 36.9 ± 1.3 years old). All subjects provided informed consent for participation.

2.2. Experimental Design

The importance of brushing of teeth and the object were defined to the subjects.
   1) Brush teeth right after getting up and before going to bed.
   2) Try to have Japanese food for breakfast and chew each mouthful more than 30 times.
   3) Drink water after having anything other than water into the mouth.
   4) Use mouthwash solution (Pro Fresh®) only twice a day, after breakfast and before going to bed.
   5) Mouthwash method.
      a) Mouthwash solution is took 7.5 cc in mouth and give the mouth a rinse.
      b) Brush it around teeth and gums with a toothbrush for one minute without spitting out a liquid.
      c) A liquid go around in mouth enough subsequently.
      d) Spit out the mouthwash.
      e) Brush it kindly toward from the depths of the tongue with Tongue Cleaner (tongue brush) which it got wet in water.
      f) Mouthwash solution is took 7.5 cc in mouth and give the mouth a rinse for 30 sec. and spit out a liquid.
   The control group is used water in place of mouthwash.
   6) Prohibit eating and rinsing the mouth for 30 minutes after using mouthwash solution.

With the results of the tests that the subjects took twice, before using mouthwash solution and after using every day (measured within 1 mouth), Intraoral gas, exhaled gas, the saliva production at rest, the ability to salivate and the buffering ability of saliva were determined.

The mouthwash solution was adjusted as below.

Solution A(water (solvent)), citric acid (pH adjuster), sodium benzoate (preservation agent), solution B(water (solvent)), sodium hypochlorite (abstergent) and sodium carbonate were put into the main bottle of mouthwash solution with sodium chlorite, shook, left for more than 30 minutes and used after spontaneously reacted.

2.3. Examination Method

2.3.1. The Measurement of Odor (Intraoral Gas and Exhaled Gas) Was Determined

The simple chromatography oral chroma (FIS Corporation, Hyogo, Japan) which was insulated from the influence of temperature and humidity and also was able to measure with a high degree of accuracy was used to measure odor as the device analysis method.

3 major components gas, hydrogen sulfide (H2S), methyl mercaptan (CH3SH) and dimethyl sulfide ((CH3)2S), were measured for 8 minutes each for intraoral gas and exhaled gas by the method of expressing concentration in increments of ppb.

As the precaution for the previous day of the test, eating food with strong smell and drinking alcohol were prohibited. As the precaution for the day of the test, to finish eating 3 hours before the test was required and smoking was prohibited. Moreover, having water or any kinds of drinks, gargling, brushing teeth and wearing anything with smell such as hair dressing and cologne were prohibited.

2.3.2. The Measurement of the Saliva Production at Rest

The secretion volume of saliva and pH determined with Saliva PFT kit (NAMITEC, LTD, Osaka, Japan) the saliva sample obtained from subjects for 3 minutes.

2.3.3. The Measurement of the Ability to Salivate

The secretion volume was measured with CAT21 Buf (Willdent Co. Ltd., Osaka, Japan) by taking the saliva sample while the subjects were chewing the chewing pellet.
2.3.4. The Measurements of the Buffering Ability of Saliva
The buffering ability of saliva was measured with CAT21 Buf (Willdent Co., Ltd., Osaka, Japan). 1 ml of saliva sample was taken while the subjects were chewing the chewing pellet and was shaken until the reagent (red) completely dissolved into it, and then was determined right after the reagent had dissolved completely and bubbles had been disappeared. The color of the liquid in the test tube was compared and the result was checked with the determination result form.

2.4. Statistical Analysis
All the data are presented as mean ± SEM. The statistical analysis was evaluated by T-test before and after using mouthwash solution. p values of <0.05 were defined as statistically significant.

3. Results
3.1. Effects of Mouthwash on Oral Malodor
The result of the intraoral gas in the oral cavity measured by using the simple chromatography was shown in Figure 1 and Table 1. The control group did not change in VSCs (Figure 1(a)). After using mouthwash solution, H2S (from 225.8 ± 28.1 ppb to 41.1 ± 8.8 ppb), CH3SH (from 72.2 ± 11.1 ppb to 7.1 ± 1.6 ppb) and (CH3)2S (from 25.5 ± 3.2 ppb to 6.2 ± 0.9 ppb) were all decreased, H2S was significantly decreased (Figure 1(b)).

The result of the exhaled gas measured by using the simple chromatography was shown in Figure 2 and Table 1.

![Figure 1](image1.png)

**Figure 1. The effects of the mouthwash solution Pro Fresh® on intraoral gas in oral cavity by oral chroma (a) control; (b) Pro Fresh® (Mean ± SE, Control: n = 5, Pro Fresh®: n = 87) **:p < 0.01 vs. pre.

![Figure 2](image2.png)

**Table 1. Intraoral gas and exhaled gas in oral cavity by oral chroma.**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>ProFresh® (n = 87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Intraoral gas (ppb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2S</td>
<td>106.4 ± 26.8</td>
<td>100.6 ± 33.3</td>
</tr>
<tr>
<td>CH3SH</td>
<td>23.6 ± 7.5</td>
<td>25.0 ± 6.1</td>
</tr>
<tr>
<td>(CH3)2SH</td>
<td>20.8 ± 3.8</td>
<td>19.2 ±4.9</td>
</tr>
<tr>
<td>Exhaled gas (ppb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2S</td>
<td>103.2 ± 23.5</td>
<td>101.0 ± 34.6</td>
</tr>
<tr>
<td>CH3SH</td>
<td>23.6 ± 1.7</td>
<td>24.2 ± 4.4</td>
</tr>
<tr>
<td>(CH3)2SH</td>
<td>24.6 ± 1.4</td>
<td>19.0 ± 4.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 vs. pre.
The control group did not change in VSCs (Figure 2(a)). After using mouthwash solution, H$_2$S (from 212.0 ± 50.4 ppb to 34.6 ± 16.6 ppb), CH$_3$SH (from 48.6 ± 28.1 ppb to 6.6 ± 3.0 ppb) and (CH$_3$)$_2$S (from 14.4 ± 4.2 ppb to 7.1 ± 1.6 ppb) were all decreased, H$_2$S and CH$_3$SH were significantly decreased, respectively (Figure 2(b)).

### 3.2. Effects of Mouthwash on Saliva

The result of the ability to salivate was shown in Figure 3 and Table 2. The control group did not change in the saliva secretion caused by stimulations (Figure 3(a)). After using mouthwash solution, the saliva secretion caused by stimulations significantly increased from 4.5 ± 0.3 ml to 5.1 ± 0.3 ml (Figure 3(b)). As for the buffering ability of saliva, the control group and mouthwash group did not change in the saliva pH (Table 2, Figure 4).

<table>
<thead>
<tr>
<th>Table 2. The saliva secretion and saliva pH.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
</tr>
<tr>
<td>Ability to salivate (ml)</td>
</tr>
<tr>
<td>Buffering ability of saliva (pH)</td>
</tr>
<tr>
<td>Saliva production at rest (ml)</td>
</tr>
<tr>
<td>Saliva pH at rest (pH)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. $^*: p < 0.05$, $^{**}: p < 0.01$ vs. pre.
The amount of saliva in rest was shown in Figure 5 and Table 2. The control group did not change in the amount of saliva in rest (Figure 5(a)). After using mouthwash solution, the amount of saliva production significantly increased from 1.3 ± 0.1 ml to 1.7 ± 0.1 ml (Figure 5(b)). As for the saliva pH in rest, the control group and mouthwash group did not change in saliva pH (Table 2, Figure 6).

4. Discussion

VSCs, the volatile nitrogen compounds and short-chain fatty acid, etc. were reported as the components of the gas in the oral cavity [12]. It is considered that VSCs is the only component that was reported to show the strong correlation between the strength of these odors and the consistency detected from the gas in the oral cavity [13]. It is also considered that a lot of bacteria in the oral cavity, especially the periodontal disease related bacteria, often have high productive performances of VSCs [14].

![Figure 4](image1.png)

*Figure 4.* The effects of the mouthwash solution ProFresh® on the buffering ability of saliva (a) control; (b) ProFresh® (Mean ± SE, Control: n = 5, ProFresh®: n = 87).

![Figure 5](image2.png)

*Figure 5.* The effects of the mouthwash solution Pro Fresh® on the amount of saliva secretion in rest (a) control; (b) Pro Fresh® (Mean ± SE, Control: n = 5, Pro Fresh®: n = 87) **:p < 0.01 vs. pre.

![Figure 6](image3.png)

*Figure 6.* The effects of the mouthwash solution Pro Fresh® on saliva pH in rest (a) control; (b) Pro Fresh® (Mean ± SE, Control: n = 5, Pro Fresh®: n = 87).
Also, it is known that the main source of the oral malodor caused by the periodontal disease comes from the tongue coat rather than VSCs which is dissolved by anaerobic bacteria produced form periodontal pockets [14] [15]. The fact that bacteria related to periodontal disease are detected with a high frequency from the tongue coat confirms this.

It is considered that to keep clean and dry the tongue is the most important because most of the food particles stagnating in the oral cavity are on its tongue. It is assumed that the mouthwash solution which has several effects on the tongue is effective to cure oral malodor.

As the feature of mouthwash solution, it is supposed to contain no hazardous chemical substances to mucous membranes such as disinfectants or synthetic surfactants at all, and the buffer solution is not supposed to acidize the oral cavity because bacteria become active if the oral cavity is acidized.

When sodium hypochlorite in solution A and citric acid in solution B are mixed together, sodium hypochlorite disappears and unsteady chlorous acid is derived. Sodium hypochlorite is dissolved immediately in the oral cavity and fresh oxygen is supplied into saliva. Consequently, bacteria of periodontal disease and so forth are not sterilized but they become disabling to act. At the same time, chloride ion suspends inside the oral cavity and becomes to stand there. This free chloride ion is bound to odorous gas such as VSCs and volatile nitrogen compounds in the oral cavity, and make a compound chloride. Oral malodor maintains the odorless condition because chloride compounds is odorless. The environment which hardly causes oral malodor is created by supplying fresh oxygen to saliva. It is proven by the research that even if oral malodor gas is produced, standing chloride ion eliminates its odors and the operation hour remains for 12 hours. It is considered that the safe environment inside the oral cavity can be kept artificially by these functions.

Moreover, although the tongue is protected by stratified squamous epithelium, the reason why it is prescribed to brush teeth to get rid of just the slime of the surface of the tongue for membrane mucosa is because 99% of saliva is water and not to remove mucin beyond necessity is more effective not to dry the tongue or cause an inflammation.

In this present study, it can be considered that the reason why the improvements of the amount of saliva in rest and the amount of saliva caused by stimulations were seen is relevant to how to use tongue brushes. To stimulate the tongue hard by tongue brushes and such causes an inflammation as membrane mucosa exfoliates and the tongue gets dried. Regarding to the results of the research about the relationship between the amount of saliva secretion and H2S:CH₃SH, it is admitted that there is a significant difference between the group that the amount of saliva in rest is lower than the standard value and the group that the amount is higher than the standard value about the averages of H₂S in the expired breath and CH₃SH concentration, however, there is also the result that is not admitted a significant difference between groups about the amount of saliva caused by stimulations. As for the relationship between the amount of saliva secretion and oral malodor, the amount of saliva secretion in rest has to be considered too [16].

Furthermore, it is reported that there is a correlation between oral malodor and the severity of periodontal disease. There is a report that the amount of VSCs are anaerobic gram-negative bacteria which are Fusobacterium known as causative bacteria of periodontal disease and Porphyromonas [19], and these bacteria dissolve desquamated epithelial cells, saliva and protein in gingival crevice fluid with the function of protease, and produce VSCs as a representative of sulfur-containing amino acid such as the derived cysteine and methionine [20] [21].

The results of the present study demonstrate that the reason why the significant differences between the first oral malodor test and the second oral malodor test could be seen can be considered that the decline in the number of causative bacteria of periodontal disease by cleaning in the oral cavity with professional cares affected. By using mouthwash solution effectively, the increase of the amount of saliva because of decreasing dryness of the tongue and the deduction of the measured value of oral malodor were seen. And it seems that they correlate each other and it will lead to create the odorless environment and increase the amount of saliva secretion.

5. Conclusions

In the present study, the experiments were conducted to verify the effect of the marketed mouthwash solution (Pro Fresh®), which contained chlorine dioxide on the inhibition of oral malodor and dryness in oral cavity.
The effect of inhibition of oral malodor and dryness in oral cavity were verified using the marketed mouthwash solution (Pro Fresh®) as mouthwash solution containing chlorine dioxide. As for residual gas in oral cavity and exhaled gas, H2S significantly decreased, respectively, and the amount of saliva secretion increased. These results suggested that mouthwash solution containing chlorine dioxide to prevent oral malodor was fully effective on inhibiting oral malodor.

References


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