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Time-dependent effects of tongue cleaning with mouthwash or mouth moisturizing gel on the number of microbes on the tongue surface of elders with care needs

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Running title: Effective tongue cleaning method

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Abstract

Objective: The purpose of this study was to investigate time-dependent change in the number of microbes on the tongue surface after tongue cleaning using a mouthwash or mouth moisturizing gel for elders fed with a feeding tube and with care needs.

Materials and Methods: Twelve elders fed through a feeding tube and with care needs participated in this prospective crossover study. There were four kinds of tongue cleaning modes as follows: 1) tongue cleaning with a mouthwash; 2) tongue cleaning with a mouth moisturizing gel; 3) tongue cleaning with water; and 4) no tongue cleaning as a negative control. The total number of microbes on the tongue surface was measured using a rapid oral bacteria detection device at baseline, immediately after cleaning, and at 1, 3 and 5 hours after cleaning to evaluate the time-dependent change for each tongue cleaning mode.

Results: There were no significant differences regarding microbial count on every measurement in negative control and tongue cleaning with water. There were significant decreases immediately after cleaning, and at 1, 3 and 5 hours after cleaning compared to baseline when the tongue was cleaned with a mouthwash. There was a significant decrease between baseline and immediately after cleaning when the tongue was cleaned with a mouth moisturizing gel.

Conclusion:

Tongue cleaning with mouth moisturizing gel decreased the number of microbes on the tongue surface immediately after cleaning, and tongue cleaning with mouthwash decreased it for 5 hours.

Introduction

The first cause of death is malignant neoplasm and the second is cardiac diseases in japan faced super-aged society. Pneumonia is the third cause of death, accounting for about 10% of the death [1]. And it is also reported that more than 80% of elderly inpatients by pneumonia were due to aspiration pneumonia [2]. The causes of community-acquired pneumonia are primarily pneumococci, mycoplasma pneumoniae and influenza virus etc. And aspiration pneumonia is mainly caused by silent aspiration of saliva containing cariogenic bacteria and periodontal pathogens as anaerobic bacteria from the mouth and pharynx[3, 4]. Many countries have an aging population, and the number of elders with care needs has also been increasing[5]. One of the causes of aspiration pneumonia in elders with care needs is the deterioration of oral hygiene that results from their inability to maintain their teeth and soft tissue in the mouth clean [4]. Because it has been reported that the incidence of aspiration pneumonia decreases with oral cleaning[6, 7], improvements in oral hygiene are an effective way to prevent aspiration pneumonia.

Yasui et al. reported that periodontal pathogens, which are a cause of aspiration pneumonia, were detected at high levels on the tongue surface of edentulous elders[8]. The tongue surface of elders with care needs are often covered with a tongue coating[9], and it is suggested that the amount of the tongue coating is related to the total number of

anaerobic bacteria on the tongue surface and in the saliva[10, 11]. Thus, the tongue surface is a reservoir of oral microbes in the saliva[12]. It has been reported that the number of anaerobic bacteria in the mouth of inpatient who were fed using a feeding tube and required daily nursing care is decreased by tongue cleaning once a day for 2 weeks with tongue brush immersed in mouthwash [13]. And it is expected that tongue cleaning improve oral hygiene and help decrease the risk of developing aspiration pneumonia.

However, it is difficult for elders with care needs to clean their mouth by themselves, and oral cleaning by caregivers may often be necessary. Because of problems such as a shortage of caregivers, it is difficult to provide sufficient oral cleaning frequently enough and for a long enough duration[14]. Therefore, a tongue cleaning method that keeps the number of oral microbes low for a long period is required.

Tongue cleaning is performed using a mechanical cleaning method that includes a tongue brush, often together with a disinfectant mouthwash and/or mouth moisturizer. Mouthwash contains a bactericidal agent, such as povidone-iodine, chlorhexidine gluconate and cetylpyridinium chloride (CPC) to provide bactericidal activity[15-18]. Mouth moisturizer contains glycerin, hyaluronic acid and/or propylene glycol for its moistening effect[19]. The number of oral microbes on the tongue surface in elders with care needs shows a more marked decrease when the tongue is cleaned with mouthwash for 2 weeks

compared with tongue cleaning with water[13]. The amount of tongue coating was reduced and the moisture level of the tongue surface was increased by performing tongue cleaning using a gel-type mouth moisturizer for 2 weeks[20]. However, the mouthwash or mouth moisturizer application methods and the frequency of their use are not established. Therefore, use of mouthwash or mouth moisturizer for tongue cleaning is clinically depended on the caregivers' experience. Our hypothesis was that tongue cleaning with mouthwash or mouth moisturizing gel decrease, and suppresses the number of oral microbes on the tongue surface longer compared to tongue cleaning with water.

The purpose of the present study was to investigate the time-dependent change in the total number of oral microbes on the tongue surface after tongue cleaning using mouthwash or mouth moisturizing gel in elders fed with a feeding tube and with care needs.

Materials and methods

Participants

It was a complete survey including all patients who were admitted to a long-term care hospital during the study period (January 2014 to August 2015), and who were fed using a feeding tube and with care needs because of cerebrovascular disease or dementia.

Participants had not been treated with antibiotics in the preceding three months, and were incapable of oral cleaning and rinsing on their own. Before the study, oral cleaning had been performed once daily by nurses using toothbrush and sterile gauze, and tongue cleaning had not been performed. Participants and their families received verbal and written information regarding the study, and provided signed consent forms prior to participation. All tests were performed in accordance with the requirements of the Revised Helsinki Declaration (Fortaleza, 2013). The study protocol was approved by the Ethics Committees of Tokyo Dental College (# 453).

Procedures

Figure 1 (a) shows that the design of this study. This study was conducted using a prospective crossover design. There were four modes depending on the tongue cleaning and participants were treated with each of four modes, with one-week washout period between each mode. The order of four modes was randomly determined. Oral cleaning was performed once a day between 9:30–10:30 am by the trained one dentist.

Figure 1 (b) shows Measurement protocol. The moisture level of the tongue surface was measured before oral cleaning. The total number of oral microbes of the tongue surface was measured five times; before oral cleaning (baseline), immediately

after cleaning, and at 1, 3 and 5 hours after cleaning. Every measurements were performed by the same dentist who performed oral cleaning at bedside.

Oral cleaning protocol

1) Tooth brushing

Tooth brushing was conducted using a toothbrush (DENT. EX Slimhead II®, Lion, Tokyo, Japan). The toothbrush was moistened with tap water, and brushing was performed using the scrubbing method. The duration of cleaning was calculated using the following formula, depending on the number of remaining teeth:

Number of remaining teeth (including remaining roots) /32 teeth × 5 minutes

2) Tongue cleaning

Tongue cleaning was performed based on estimated four modes: 1) tongue cleaning using mouthwash (MW); 2) tongue cleaning using mouth moisturizing gel (MG); 3) tongue cleaning with water (W); and 4) no tongue cleaning as negative control (NC);, using a tongue brush (Freshmate soft®, DENT CARE Inc., Osaka, Japan). Tongue cleaning was performed 5 times on each side for 1 minute, from the anterior part of the terminal sulcus to the tip of the tongue.

For the MW mode, tongue cleaning was performed using a tongue brush moistened with mouthwash (ConCool mouth rinse®, Weltec, Osaka, Japan). For the MG mode, 1 g of mouth moisturizing gel (Refrecare H®, EN Otsuka Pharmaceutical, Iwate, Japan) was evenly applied between the anterior part of the terminal sulcus and the tip of the tongue, followed by tongue cleaning using a dry tongue brush. For the W mode, tongue cleaning was performed using a tongue brush moistened with tap water. For the NC mode, tongue cleaning was not performed although tooth brushing was performed. Main ingredients of mouthwash and mouth moisturizing gel were shown in Table 1.

The mouthwash used in the present study included bactericidal CPC (Table 1). Although it is reported that the povidone-iodine and chlorhexidine used in mouthwash frequently exert strong bactericidal activity[15], there have also been reports of anaphylactic shock after the use of povidone-iodine and chlorhexidine in elders[21]. Therefore, the use of these ingredients is not recommended for elders with care needs. CPC demonstrates both bactericidal activity and a good safety profile[22, 23], and is considered to be safe for use in the participants of the present study.

The mouth moisturizer used in the study was a gel-type product, with intermediate-level viscosity among the commercially available moisturizers in Japan[24]. It contains hinokitiol as an antimicrobial ingredient similar to the mouthwash used in this study

(Table 1). This intermediate-viscosity gel-type mouth moisturizer was assessed in the previous study[20] and in our preliminary study (data not shown), which showed a longer period of reduction in the total number of oral microbes on the tongue surface after tongue cleaning, compared with spray or liquid-type moisturizers.

3) Wiping

Because the participants could not gargle, wiping was performed instead. Oral microbes which were spreaded in the mouth by tooth brushing and tongue cleaning were wiped off using oral wet wipes(Oral care Wetty®, Wakodo, Tokyo, Japan). Main ingredients of the wipes were shown in Table 1. For the W, MW, and MG modes, the tongue surface was wiped three times, from the terminal sulcus to the tip of the tongue, the teeth were wiped once unicursally from the buccal to lingual side, and the oral mucosa excluding the tongue surface (sublingual area, palate, and oral vestibule) was wiped once. For the NC mode, the wipe procedure was performed but without wiping the tongue.

Evaluation

The moisture level of the tongue surface was measured using an oral moisture measurement device (Mucus®, Life, Saitama, Japan). This oral moisture-checking device

measures electrostatic capacity that reflects not only the water content of the oral mucosal surface but also the intramucosal water content to 50 μm depth. The numerical data are expressed as 3-digit numbers, ranging from 0.00 to 99.8. The value strongly correlates positively with the actual gravimetric moisture percentage, with a correlation coefficient of 0.99, and is a relative measure of water content, rather than the actual moisture percentage. To perform measurements, a sensor of this device was brought into vertical contact with the surface of the tongue 10 mm from the tip of the tongue[25]. Measurement was performed three times, and the median value was used.

The total number of oral microbes on the tongue surface was measured using a rapid oral bacteria detection device (Bacterial Counter DU-AA01NP-H, Panasonic Health Care, Tokyo, Japan). This device was developed by applying Panasonic's the DEPIM (DiElectroPhoretic Impedance Measurement) method, consisting of dielectrophoresis and impedance measurement. Aspiration pneumonia is mainly caused by silent aspiration of saliva containing anaerobic bacteria from the mouth and pharynx [4]. Some study reports that anaerobic bacteria are decreased by oral cleaning and the risk of aspiration pneumonia decreases [7, 13]. It is also reported that many anaerobic bacteria are detected from tongue surface [8]. DEPIM method used for counting the total number of microbes showed a high correlation between anaerobic culture methods using oral samples

containing a mixture of various bacterial species [26]. In this study, the total number of oral microbes on the tongue surface was measured because it is suggested that anaerobic bacteria including fungus causing aspiration pneumonia decreased when the total number of microbes decreased. Before sample collection, 2 ml of tap water was sprayed onto the tongue surface. A sterile swab was dipped in tap water, and the sample was taken by swabbing anteriorly 5 times from the anterior part of the terminal sulcus on the median groove of the tongue. The used swab was then inserted into a disposable cup with pure water, and the total number of oral microbes in the sample was measured.

The viscosity of tap water, the mouthwash and the mouth moisturizing gel used for tongue cleaning was measured using a viscometer (VM-10A-L/KN3312485, Sekonic Corporation, Tokyo, Japan). Measurements were performed three times each, and the mean was calculated.

Statistical analysis

Because the number of participants was limited, non-parametric test was performed addition to crossover design. The total number of oral microbes on the tongue surface before oral cleaning (baseline) was compared using the Kruskal-Wallis test, followed by the Steel-Dwass test. The total number of oral microbes on the tongue surface at every

measurement was compared using Friedman's test followed by Dunnett's test. The microbial counts were logarithmically transformed and were used for statistical analysis. A level of 0.05 was considered significant. Statistical analysis was performed using SPSS software for Windows, version 21 (IBM Corp., Armonk, NY, USA).

Results

Participants were 12 elders (mean age, 80.1 ± 8.3 years; 7 males and 5 females).

The mean moisture level on the tongue surface before oral cleaning was 6.4 ± 7.0, the median was 8.7 and the range was 1.7-19.3.

Figure 2 shows the total number of oral microbes at baseline. The overall mean for all modes was $2.5 \times 10^7 \pm 0.7 \times 10^7$ CFU/ml, and there was no significant difference among any of the modes. Figures 3 (c) and (d) show the time-dependent change in the total number of oral microbes on the tongue surface for the W and NC modes, respectively. No significant differences were observed between any of the measurements. Figure 3 (a) shows the time-dependent change in the total number of oral microbes on the tongue surface for the MW mode. Significant decreases were observed immediately after oral cleaning ($P = 0.000$), and 1 hour ($P = 0.000$), 3 hours ($P = 0.000$) and 5 hours ($P = 0.019$) after oral cleaning compared with before oral cleaning. Figure 3 (b) shows the time-

dependent change in the total number of oral microbes on the tongue surface for the MG mode. A significant decrease was observed immediately after oral cleaning compared with before oral cleaning ($P = 0.000$), but there were no significant differences at 1, 3 or 5 hours after cleaning. The value of coefficient of variation of observed data in this study was between 0.021 and 0.091 (mean, 0.053; standard deviation, 0.017), and the variation of data was very low.

The viscosity of the materials used for tongue cleaning was 0.88 ± 0.01 mPa·s for water, 7.27 ± 0.28 mPa·s for mouthwash and 396 ± 11.79 mPa·s for mouth moisturizing gel.

Discussion

From our results, hypothesis that tongue cleaning with mouthwash or mouth moisturizing gel decrease, and suppresses the number of oral microbes on the tongue surface longer compared to tongue cleaning with water was approved.

There were significant decreases observed in the total number of oral microbes on the tongue immediately after cleaning compared with before oral cleaning for the MW and MG modes, but there were no differences for the NC and W modes. The tongue surface in elders with care needs is dry[27], and a large amount of tongue coating and

keratinocyte-derived products are often found[28]. The mean moisture level of the tongue surface before oral cleaning was 6.4 ± 7.0. Because the index for dry mouth is defined as lower than 27[27], the present participants were considered to have dry mouth. Because elders with care needs tend to have a dry mouth, the tongue coating and keratinocyte-derived products on the tongue surface are also dry, making the coatings difficult to remove. It is thought that the moisture level of the tongue surface is increased using a mouthwash and a mouth moisturizing gel with a moisturizing ingredient, causing the tongue coating or keratinocyte-derived products to soften and they are more easily removed. In contrast, the number of microbes was not decreased immediately after oral cleaning using the W mode. Because water does not contain a moisturizing agent, the transpiration of water is higher than that for mouth moisturizing gel and mouthwash[19]. It might be difficult to soften the tongue coating or keratinocyte-derived products on the tongue surface. Biofilm on the tooth surface and the tongue surface is also cleared by oral cleaning. Previous reports have shown that wiping after oral cleaning has a similar effect of eliminating oral microbes by rinsing and suctioning the mouth in elders with care needs[29]. Our results showed that there was no increase of the number of oral microbes immediately after oral cleaning. This may be because the whole mouth was wiped at the end of oral cleaning in this study.

Although the number of oral microbes returned to pre-oral cleaning levels after oral cleaning using the MG mode, a similar return was not observed until 5 hours after oral cleaning using the MW mode. The tongue surface is covered with tongue papillae including filiform, fungiform, foliate and circumvallate types. The tongue surface is rough compared with other oral mucosae because of these papillae, and debris can attach to its surface. Additionally, the deeper region between the tongue papillae is an anaerobic environment that provides a favorable environment for bacteria. It is thought that a solution with lower viscosity has the higher the extent of osmosis. The relatively lower viscosity of the mouthwash used in the present study compared with the mouth moisturizing gel may have osmose and reached deeper regions between the papillae. It is expected that oral microbes were easily removed because the dry biofilm in the deeper regions between the papillae were moistened. The results of our preliminary study showed that the number of oral microbes can be kept low by maintaining a high moisture level for the tongue surface after tongue cleaning. Additionally, it was also shown that tongue cleaning with a gel-type mouth moisturizer keeps the number of oral microbes low regardless of whether it has a bactericidal ingredient. The number of oral microbes returned to pre-tongue cleaning levels one hour after cleaning, despite the inclusion of bactericidal ingredients in the mouth moisturizing gel for the MG mode. It is suspected

that the bactericidal ingredients did not infiltrate into the deeper region between the papillae because of its high viscosity, and therefore, it was removed when the tongue surface was wiped. In contrast, when tongue cleaning was performed using a low-viscosity mouthwash, it is suspected that a higher moisture level for the tongue surface was maintained because of infiltration of the moisturizing ingredients between the papillae after tongue cleaning. Therefore, this may have led to a longer duration of oral microbial growth inhibition. Future studies are needed because the moisturizing ingredients and bactericidal ingredients contained in the mouthwash used in this study could affect the decrease in the number of microbes between the papillae.

The results of the present study suggested that the number of oral microbes on the tongue surface is decreased for a longer duration after tongue cleaning when using the mouthwash of lower viscosity compared with the mouth moisturizing gel, allowing better infiltration between the papillae in elders with care needs. It is believed that methods using mouthwash are more effective for cleaning the tongue in the dry mouth of elders who undergo tube feeding and who have care needs. In the present study, we showed that tongue cleaning with mouthwash keeps the number of microbes on the tongue surface low for a longer period. It is expected that applying mouth moisturizing gel on the tongue surface after tongue cleaning extends the effect of cleaning even more by preventing

mouthwash transpiration.

Conclusion

In this study, it is clarified that tongue cleaning with mouth moisturizing gel decreased the number of microbes on the tongue surface immediately after cleaning and that with mouthwash decreased it for 5 hours although that with water did not decreased it.

The results of the present study may help to establish an effective tongue cleaning method to reduce the risk of developing aspiration pneumonia in elders with care needs.

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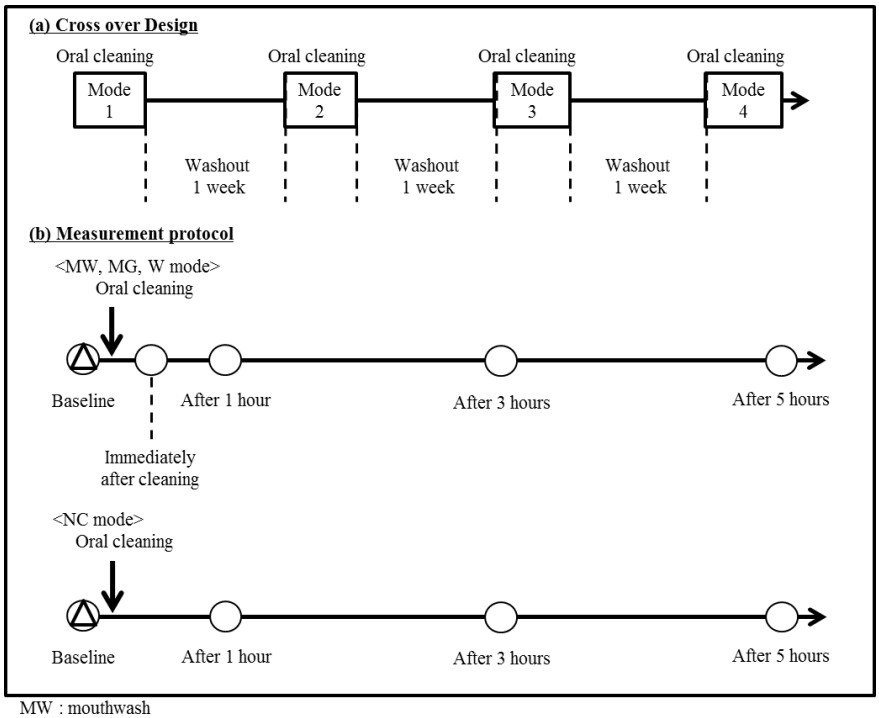
Table 1. Main ingredients/Components of Materials

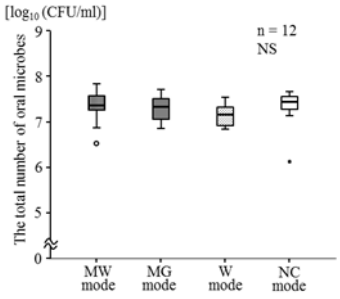
Material	Main ingredients/Components
Mouth wash	water, propylene glycol, sorbitol, xylitol, cetylpyridinium chloride
Mouth moisturizing gel	water, glycerin, haluronate sdium, xylitol, hinokitiol
Oral wet wipe	water, ethanol, propylene glycol, glycerin

Figure 1. Protocol of Cross over study



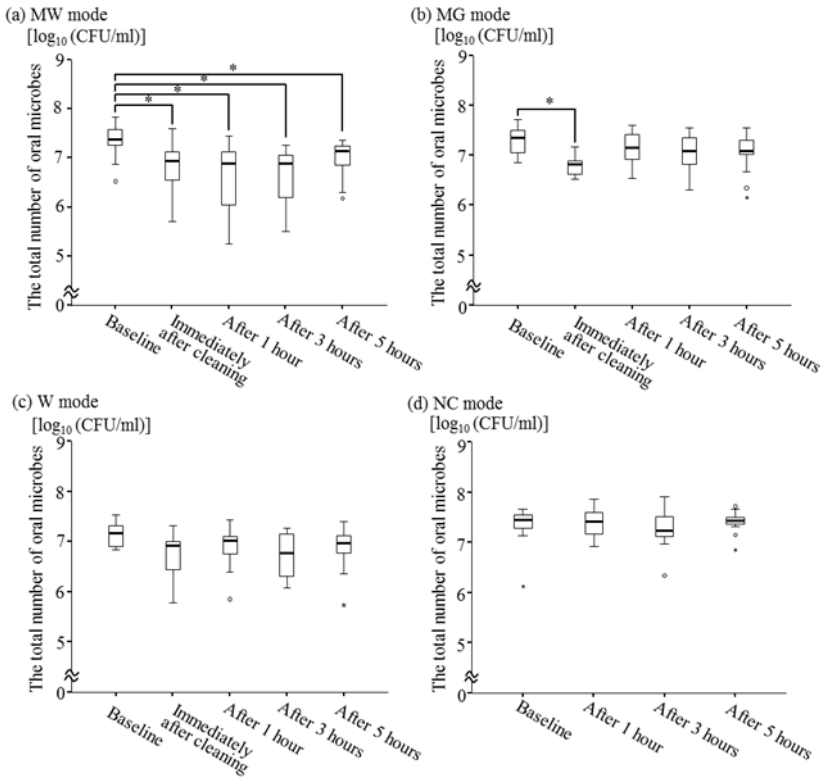
MW : mouthwash
MG : mouth moisturizing gel
W : water
NC : negative control
○ : measurement of the number of microbes on the tongue surface
△ : measurement of the moisture level on the tongue surface

Figure 2. Total number of oral microbes on the tongue surface before tongue cleaning



MW : tongue cleaning with a mouthwash
MG : tongue cleaning with a mouth moisturizing gel
W : tongue cleaning with water
NC : no tongue cleaning as a negative control
CFU : colony forming unit
NS : not significant with Kruskal-Wallis test, followed by the Steel-Dwass test

Figure 3. Time-dependent change in the total number of oral microbes on the tongue surface



MW : tongue cleaning with a mouthwash
MG : tongue cleaning with a mouth moisturizing gel
W : tongue cleaning with water
NC : no tongue cleaning as a negative control
CFU : colony forming unit
*P>0.05 ; Friedman's test followed by Dunnett's test
NS : not significant

Figure Legends

Figure 1.

(a) Cross over Design

The order of the four tongue cleaning modes was randomly determined, as follows: MW, MG, W and NC. The washout period was one week between each mode.

(b) Measurement protocol

Moisture level on the tongue surface was measured before oral cleaning and the total number of oral microbes on the tongue surface was measured 5 times: before oral cleaning (baseline), immediately after cleaning, at 1, 3 and 5 hours after cleaning. Measurement immediately after cleaning was not performed for the NC mode because tongue cleaning was not performed.

Figure 2. Total number of oral microbes on the tongue surface before tongue cleaning

The total number of oral microbes on the tongue surface before tongue cleaning was compared using the Kruskal-Wallis test, followed by the Steel-Dwass test.

⋆, extreme value; ◦, outlier

The horizontal line that forms the top of the box is the 75th percentile. The horizontal line that forms the bottom is the 25th percentile. The horizontal line that intersects the box is the median. Horizontal lines above and below the box, called whiskers, represent maximum and minimum values.

Figure 3. Time-dependent change in the total number of oral microbes on the tongue surface

The total number of oral microbes on the tongue surface at each measurement was compared using the Friedman’s test followed by Dunnett's test.