Risk factors for the development of oral bacteria in workers according to oral environment

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Abstract  This research examined the oral environmental factors to identify the risk factors for oral bacteria detection. This study comprised of 60 office workers aged between 20 and 65 years, and was performed from January 15 to February 28, 2015. The study variables measured were the stimulated and unstimulated salivary flow rates, salivary buffering, saliva pH, dry mouth at the dorsum of the tongue and the sublingual region, halitosis, and the degree of tongue-coating as oral environmental factors. To identify the presence of oral bacteria, pathogens were detected by extracting the gDNA of the resting salivary flow rate. The risk of S.mutans detection was 15 times higher with smokers, 1.3∼1.6 times higher when the resting or stimulated salivary flow rate was reduced by 1 mm. The risk of P.intermedia detection was 13 times higher in smokers, 4.3 times higher as the severity of oral dryness was lowered, and 4 times higher for adults with a tongue coating than those without. In addition, the risk of detecting TM7 was 5.5 times higher as sublingual dryness was decreased by 1mm. The oral bacterial count will be reduced considerably by smoking cessation education and habits that facilitate a salivary flow rate. Furthermore, adults with good and well-managed dental hygiene are anticipated to have less oral bacteria and fewer dental diseases.

Keywords : TM7 phylum, Oral environment, P.Intermedia, S.mutans

1. Introduction

The resident flora exists according to micro-environment including buccal mucosal surface, tongue surface, gingival sulcus, saliva and others in the oral cavity. The bacterial species and ratios of oral resident flora are influenced by individual growth, tooth eruption and loss, types of food intake, saliva composition, dental hygiene, presence of disease and others[1-2]. Since the mouth is connected to outer
environment, oral bacteria are believed to be living in a very dynamic environment[2]. Moreover, the oral cavity is the part of body in which eating, talking and other dental hygiene activities take place, and this has a substantial impact on bacterial growth and activities. The oral cavity is also greatly influenced by dietary pattern, age and health state, and constant changes may be manifested in pH, saliva composition and sodium concentration inside the mouth[3]. Bacteria survivorship is influenced by these significant changes including bacteria susceptible to stimulation.

Every human mouth has unique salivary flow rate(SFR) and microbial composition, and every individual has distinctive plaque composition and formation. Therefore, oral health status can be clinically determined based on plaque biomass, pH, microbial reaction and others. In addition, this can explain why a person is more prone to disease than the other person despite the same dental hygiene habits[4]. Dental diseases change microflora composition.

Dental caries increase the number of Streptococcus mutans(S.mutans) and Lactobacilli, and periodontal diseases accelerate the proliferation of gram-negative bacteria[1]. Dental caries and periodontal diseases are the most prevalent oral infectious diseases that are commonly caused by bacteria. For this reason, identify oral bacteria is important in understanding pathogenic mechanism and preventing and managing dental diseases[5].

S.mutans is most profoundly associated with tooth decay among mutans streptococci and found in the dental plaque of caries region and saliva[6]. periodontal disease is the leading cause for tooth loss by generating periodontal tissue destruction and bone resorption due to complex bacterial infection. Prevotella intermedia (P.intermedia) is one the main pathogen for periodontal diseases and predominantly present with in periodontal cysts in adult periodontitis. In addition, this pathogen is associated with acute necrotizing ulcerative gingivitis and pregnancy gingivitis[1].

TM7 phylum(TM7)'s increased prevalence in periodontal sites, TM7 is now associated with periodontitis and it is believed[7-8]. Additionally, TM7 has been associated with cystic fibrosis, inflammatory bowel disease, and vaginosis[9-11], like this periodontitis, and DNA analysis shows that this bug has the ability to create many toxins.

The pathogens of periodontal diseases are related with the presence and severity of periodontal diseases[12]. Detecting potential pathogens and examining their inflammatory response are very important in diagnosing adult dental health.

Gingivitis, periodontitis, and chronic necrotizing ulcerative gingivitis, approximately 35% of adults are being suffered by these, are also type of diseases caused by bacterial infection; in this, often P. intermedia and S. mutans are two common observed bacterial strains[13]. Thus far, most investigations have focused on associations between oral diseases and smoking status[14-15], relationships between salivation and oral diseases[1,3-6,16], as well as periodontal diseases and oral bacteria in contexts of disease causing oral bacteria[1,9-11]. Further, even though there are studies investigating with regards to relationship between oral bacteria and respective oral disease types, no study have utilized saliva as a diagnostic tool for oral inflammatory diseases including dental caries, periodontal disease, and gingivitis.

oral environment and bacteria are profoundly associated[4,5]. Studying risk factors for oral bacteria detection is very meaningful in preventing oral diseases from oral public health perspectives.

Therefore, this study examined oral environmental factors and the presence of oral bacteria in adult workers. This investigation aims to establish a reference base to diagnose and prevent oral diseases by identifying risk factors of oral bacteria.

2. Materials and Methods

2.1 Materials
This study comprised 60 office workers aged
between 20 and 65 years, and performed from January 15 to February 28, 2015. All subjects consented and were fully informed about research procedures. This study was conducted after gaining Institutional review board (IRB) approval (BUIRB-201410-HR-0011). In this research, 20 men (33.3%) and 40 women (66.7%) were enrolled as subjects, and their mean age was 33.8 years. There were 37 non-smokers (61.7%) and 23 smokers (38.3%). With respect to alcohol consumption, 20 (33.3%) were non-drinkers, 40 (66.7%) were drinkers. Regarding systemic diseases, 47 were healthy subjects (71.6%) and 17 (28.4%) were currently taking medication for systemic diseases. Systemic diseases were grouped into diabetes, hypertension, cardiovascular disease, heart disease, and liver disease.

2.2 Methods

To identify bacteria species, we measured unstimulated and stimulated SFRs. To identify oral environmental factors salivary buffering capacity, saliva pH, dryness at the dorsum of the tongue and the sublingual region, halitosis, and the degree of tongue-coating.

2.2.1 Saliva Collection

To examine oral bacteria, a subject’s saliva sample was collected into a paper cup for 5 min without applying any stimulation and measured its amount in ml. A saliva flow of less than 0.7ml was defined as “small”, and a saliva flow of greater than 0.8ml was defined as “normal”. Saliva samples were collected between 12-4 pm when saliva flow is most active, and 2 hours after tooth brushing. For DNA extraction, obtained plaque sample was put into a test tube and genomic DNA was extracted in the laboratory. To measure stimulated SFR, saliva samples were collected into measuring cups for 5 min after asking subjects to chew paraffin wax for 1 min to facilitate salivation. The volume of saliva was measured using cups’ markings. A saliva flow of less than 5.0ml was classified as “small”, and a saliva flow of greater than 5.1ml was classified as “normal”. A higher volume of saliva implies a healthier oral status.

2.2.2 Salivary Buffering Capacity

Collected stimulated saliva was dropped on a piece of test strip. For better absorption of saliva, the test strip was placed in a vertical position at an angle of 90°, and then the color change of test papers were observed. Green color was given 4 points, 3 points for green/blue color, 2 points for blue color, 1 point for red/blue color, and 0 point for red color. Salivary buffering capacity was evaluated by calculating the total score. The score ranged between a minimum score of 0 to a maximum score of 12. A higher score indicates a greater salivary buffering capacity. This study defined a score of less than 9 as “low” and a score between 10-12 as “normal”.

2.2.3 Salivary pH

A pH test strip was soaked into collected saliva for 10 sec, and then the color of test strips was compared to pH indicators. A pH of 5.0-5.8 was defined as “acidic”, 6.0-6.6 as “normal”, and 6.8-7.8 as “healthy”. In this study, pH was classified into categories of greater than 6.8 and less than 6.6. A higher pH means a healthier status.

2.2.4 Assessment of Mouth Dryness with Absorbent Paper Strip

Absorbent paper strips (Wet-test, Kiso, Japan) were placed at the dorsum of the tongue and the sublingual region using forceps, and then placed in a vertical position for 10 sec each. Absorbed saliva was measured in mm. A larger volume of saliva absorbed by the paper strip indicates a better health state.

2.2.5 Assessment of Halitosis

Halitosis was measured by a portable halitosis detector (TANITA HC-212M, Japan), and these objective values were classified into 6 scales (0-5 levels). The severity of odor was classified into 0 as no odor, 1 as barely noticeable, 2 as slight noticeable, 3
as moderate, 4 as strong, and 5 as extremely strong. We defined 0-1 levels as absence of halitosis and 2-5 levels as presence of halitosis.

2.2.6 Tongue Coating Index (TCI)

The tongue from tip to root was divided into thirds lengthwise and crosswise to a total of 9 sections, and then scores were given according to the presence of dental plaque on lingual surfaces in each section. Zero point was defined as no plaque group, 1 point as group with thin plaque and lingual papilla and 2 points as group with thick plaque and without lingual papilla. The TCI score ranged between a minimum score of 0 to a maximum score of 18. A higher TCI score indicates a greater amount of tongue coating.

2.2.7 Extraction of Bacterial Genomic DNA and PCR

Species-specific PCR primers were used to detect *S.mutans* ATCC25175, *P.intermedia* ATCC25611, and *TM7 phylum* designed in this study. To obtain the optimum annealing temperature of any primer pair, denaturation and extension were consistently carried out at 95ºC for 30sec and 72ºC for 1 min. PCR was performed at 59-72ºC for 1 min for annealing. For the verification of manufactured PCR primers, procedures were done on a PTC-100 thermocycler (MJ Research Ins., Watertown, MA, USA) using AccuPower PCR Premix (Bioneer Corp., Daejeon, Korea). To make a 25ul of PCR mixed solution, a 5uM of forward and reverse primers each and a 1ug of genomic DNA of each strain were added. Early denaturation was done at 95ºC for 5 min, and next denaturation was conducted at 95ºC for 30sec, followed by annealing at 58-72ºC for 1 min and extension at 72ºC for 1 min. This process was repeated for 35cycles for amplification, and then extension was finally conducted at 72ºC for 10 min. Nested PCR technique was performed for *S.mutans* and *TM7*. In the primary PCR, primer pair was used to amplify bacterial 16S rDNA sequences from all bacterial species. Afterward, the secondary PCR was carried out with species-specific PCR primer pair. Predicted sizes were *S.mutans* 356bp, *P.intermedia* 300bp, and *TM7* 1000bp. Electrophoresis was conducted on final reactants using 0.1% agarose gels to determine DNA amplification(Fig 1).

2.3 Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics 20.0(SPSS Inc. Chicago, IL, USA). Frequency analyses were done to determine the presence of bacterial species in the oral cavity. A chi-square test was used to verify the association of oral bacteria detection with drinking, smoking, systemic diseases, oral environmental factors (stimulated or unstimulated SFR, salivary buffering capacity, pH, halitosis, and tongue-coating). Moreover, a logistic regression analysis was performed to verify the risk factors of oral bacteria according to oral environmental factors.
3. Results

3.1 Detection of Oral Bacteria

The results of detected oral bacteria are shown in Figure 2. There were 38 subjects with *S. mutans*, 46 with *P. intermedia*, 9 with TM7, 32 with *S. mutans* and *P. intermedia*, 6 with *S. mutans* and TM7, 9 with *P. intermedia* and TM7, 6 with all three bacteria species, and 8 without any bacteria.

![Fig. 2. Number of oral bacteria detection(multiple response)](image-url)

### Table 1. Association of Oral Bacteria Detection with Oral Environmental Factors

<table>
<thead>
<tr>
<th></th>
<th>No (%)</th>
<th>Yes (%)</th>
<th>X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>6(30.0%)</td>
<td>14(70.0%)</td>
<td>0.574</td>
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<tr>
<td></td>
<td>Female</td>
<td>16(40.0%)</td>
<td>24(60.0%)</td>
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<tr>
<td>Age</td>
<td>20-29</td>
<td>11(42.3%)</td>
<td>15(57.7%)</td>
<td>0.645</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>6(33.3%)</td>
<td>12(66.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥40</td>
<td>5(31.3%)</td>
<td>11(68.7%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
<td>19(50.0%)</td>
<td>19(50.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3(13.6%)</td>
<td>19(86.4%)</td>
<td>1(4.5%)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>No</td>
<td>7(35.0%)</td>
<td>13(65.0%)</td>
<td>0.036</td>
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<td></td>
<td>Yes</td>
<td>15(37.5%)</td>
<td>25(62.5%)</td>
<td></td>
</tr>
<tr>
<td>Systemic disease</td>
<td>No</td>
<td>16(37.2%)</td>
<td>27(62.8%)</td>
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<tr>
<td></td>
<td>Yes</td>
<td>15(35.3%)</td>
<td>11(64.7%)</td>
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<tr>
<td>Unstimulated saliva rate</td>
<td>Normal</td>
<td>7(38.9%)</td>
<td>11(61.1%)</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>15(35.7%)</td>
<td>27(64.3%)</td>
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<tr>
<td>Stimulated saliva rate</td>
<td>Normal</td>
<td>8(57.1%)</td>
<td>6(42.9%)</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>14(30.4%)</td>
<td>32(69.6%)</td>
<td></td>
</tr>
<tr>
<td>Buffer capacity</td>
<td>Normal</td>
<td>10(43.5%)</td>
<td>13(56.5%)</td>
<td>0.745</td>
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<td></td>
<td>Low</td>
<td>12(24.4%)</td>
<td>25(75.6%)</td>
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<tr>
<td>pH</td>
<td>≥6.8</td>
<td>19(38.0%)</td>
<td>31(62.0%)</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>≤6.6</td>
<td>3(10.0%)</td>
<td>7(90.0%)</td>
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<tr>
<td>Halitosis</td>
<td>No</td>
<td>13(43.3%)</td>
<td>17(56.7%)</td>
<td>1.148</td>
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<td></td>
<td>Yes</td>
<td>9(30.0%)</td>
<td>21(70.0%)</td>
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<tr>
<td>Tongue-coating</td>
<td>No</td>
<td>14(38.9%)</td>
<td>22(61.1%)</td>
<td>0.191</td>
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<td></td>
<td>Yes</td>
<td>8(33.3%)</td>
<td>16(66.7%)</td>
<td></td>
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</tbody>
</table>

*p* = 0.05 to determined by chi-square test

3.2 Association of Oral Bacteria Detection with Oral Environmental Factors

The results of oral environmental factors and oral bacteria examination are as follows (Table 1). *S. mutans* had a significant difference with smoking. A greater number of *S. mutans* were found in smokers than in non-smokers. The number of *S. mutans* was greater for males especially in their 40s, smokers, non-drinkers, and adults with systemic disease, low resting or stimulated SFR, low salivary buffering capacity, pH of less than 6.6, halitosis and tongue coating. However, no significant difference was found. *P. intermedia* showed a significant difference with age, smoking, patients with systemic diseases and salivary buffering capacity. A greater number of *P. intermedia* were found in adults in their 40s, smokers, patients with systemic disease, and subjects with lower salivary buffering capacity than healthy persons. The number of *P. intermedia* was greater for males, drinkers, and adults with low resting or stimulated SFR, pH of less than 6.6...
6.6, halitosis and without tongue coating. TM7 had a significant difference with age and systemic disease. A higher number of TM7 were detected in adults in their 20s and those without systemic disease. The number of P.intermedia was higher for males, smokers, drinkers, and adults with normal resting or stimulated SFR, normal salivary buffering capacity, pH of less than 6.6, halitosis and tongue coating. However, no significant difference was found.

3.3 Oral Environmental Risk Factors Affecting Oral Bacteria Detection

Oral environment risk factors affecting oral bacteria detection are as follows (Table 2). S.mutans exhibited a significant difference with smoking, resting SFR and stimulated SFR. The risk of S.mutans being detected in saliva was 15 times higher for smokers than for non-smokers. The risk of S.mutans detection was 1.6 times higher as resting SFR was reduced by 1mm, while the risk of S.mutans detection was 1.3 times higher as stimulated SFR was reduced by 1mm.

P.intermedia had a significant difference with smoking, sublingual dryness, and tongue-coating. The risk of P.intermedia being detected in saliva was 13.6 times higher for smokers than for non-smokers.

The risk of P.intermedia detection was 4.4 times higher as the severity of sublingual dryness was reduced and 4 times higher for adults with tongue-coating than those without. In addition, TM7 had a significant difference with sublingual dryness, and the risk of detecting TM7 was 5.5 times higher as sublingual dryness was decreased by 1mm.

4. Discussion

This study aimed to examine oral environmental factors and identify risk factors affecting oral bacteria detection in oral environment.

First, The results of this study show that S.mutans, an etiological agent of tooth decay, is dominantly found in smokers. The risk of S.mutans being detected

| Table 2. Oral Environmental Risk Factors Affecting Oral Bacteria Detection |
|-----------------------------|-----------------|-----------------|-----------------|
| Smoking                     | S. mutans       | P. intermedia   | TM7             |
| Adj ORa(95% Cl)             | No Detection    | No Detection    | No Detection    |
| Smoking                     | 15.15(2.36-97.26)** | 13.58(0.86-21.74)* | 0.96(0.85-1.09) |
| Drinking                    | 0.44(0.09-2.07)  | 0.48(0.05-4.36)  | 2.77(0.17-44.91) |
| Unstimulate saliva rate     | 1.64(0.99-2.73)** | 0.73(0.42-1.27)  | 1.12(0.66-1.89)  |
| Stimulate saliva rate       | 0.79(0.62-1.03)* | 1.21(0.88-1.67)  | 1.17(0.90-1.52)  |
| Dorsum of tongue            | 0.55(0.07-4.20)  | 0.86(0.02-37.67) | 6.59(0.27-15.14) |
| Floor of tongue             | 1.14(0.49-2.62)  | 0.23(0.05-0.98)** | 0.18(0.02-1.16)* |
| Buffer capacity             | 1.08(0.67-1.74)  | 0.57(0.25-1.33)  | 0.89(0.43-1.86)  |
| pH                          | 0.45(0.04-4.52)  | 1.57(0.03-65.62) | 0.49(0.01-23.73) |
| Halitosis                   | 2.95(0.36-23.72) | 0.02(0.00-0.83)  | 0.20(0.01-6.04)  |
| Tongue coating index        | 0.89(0.37-2.11)  | 4.02(0.80-20.23)** | 2.03(0.51-8.03)  |

The CI means confidence interval

*p<0.05, **p<0.01 to determined by logistic regression analysis, *p=0.05 to one-tailed.

The adjustment for gender and age
in the mouth is 15 times higher.

In previous studies the caries causing bacteria in the oral environment were approximately worse in smokers due to \textit{S.mutans} distribution being dominant[14, 17-18]. it was that \textit{S.mutans} where three times higher in smokers[15]. The results showed a higher risk than previous studies. it was considered that when the oral environment is deteriorating dental caries, it is directly related to distribution and \textit{S.mutans} due to smoking.

Moreover, the likelihood of \textit{P.intermedia} being detected in saliva is 13 times higher for smokers than for non-smokers. There have been several studies that have shown that the risk of periodontal disease is 1.63 times higher[19], and the risk of periodontitis is 2.5-6 times higher[20]. The likelihood of periodontitis incidence and deterioration is 5-7 times higher in heavy smokers[21]. This outcome implies that smoking has negative effects on periodontal disease, and a large number of epidemiological studies have proposed that there is a strong association of smoking with the prevalence and severity periodontal disease[22].

The risk of detecting bacteria related with oral diseases has been found to be higher in smokers. Adults with oral bacteria are more likely to get caries and periodontal disease. Smoking serves as an important environmental factor in the development of several oral diseases[23], and is a contributory factor in the increase of bacterial distribution in the oral cavity. The effect of smoking on bacterial species varies depending on investigators and research methods. However, oral environment changes caused by smoking have potential to change bacterial species.

Second, According to the finding of this study, the risk of \textit{S.mutans} detection is identified to be 1.3-1.6 times higher in adults with a lower SFR in comparison to the healthy individuals. The risks of \textit{P.intermedia} and \textit{TM7} being detected in saliva were about 4 and 5 times higher, respectively, in adults with low sublingual dryness.

It has been known that salvation and distribution of oral bacteria are closely related[1,3-5]. In particular, given the previous studies demonstrating that occurrence rate of caries is negatively correlated with amounts of salvation of dorsum and hypoglossal tongue because of less sugar contents therein[24] and patients with gingivitis had more oral \textit{P. intermeia}, it seems reasonable to believe[25] that salvation is one of decisive factors for oral diseases.

In addition, there is a high possibility that reduced salivation has influenced oral environment, leading to inflammation such as gingivitis since \textit{TM7} has been detected. Therefore early colonization of \textit{TM7} and \textit{P.intermedia} represents a risk to the future progress of periodontal disease. Thus proving that \textit{TM7} and \textit{P.intermedia} causes of oral bacteria[26].

Third, The risk of \textit{P.intermedia} detection has been reported to be about 4 times higher in adults with a greater amount of tongue-coating, indicating the importance of tongue-coating management. In fact, there are many reports on amount of tongue-coating and its high relationship with halitosis. The amount of tongue-coating was six times greater in comparison with gingivitis halitosis.[27]. Halitosis and tongue-coating can be prevented by increasing saliva amount with artificial saliva, removing tongue-coating, treating periodontal disease or oral inflammation, educating people with proper tooth and tongue brushing, and taking fresh fruits and vegetables including low-fat foods.

Finally, a greater number of \textit{P.gingivalis}-positive sites have been observed in patients with systemic diseases. In patients with systemic diseases, there has been found a variety of mechanisms increasing the prevalence or severity of periodontitis[28]. Systemic diseases have increased the incidence and severity of periodontal diseases by limiting physical movement and making difficult to maintain good oral hygiene in some patients[29]. \textit{P.gingivalis} and \textit{P.intermedia} are found to be involved as inflammatory mediators in relation to systemic and periodontal diseases. Those bacterial species play primary roles in the development of several diseases including diabetes[30], rheumatic
disorders[31], cardiovascular diseases[32], pneumonia[33] and others. They have been assumed to perform a contributory function in inflammatory-related mechanism. In contrast, a higher level of TM7 has been detected in healthy individuals. They younger the age, the higher the level of TM7. TM7 is uncultured bacterium commonly found in case s of inflammatory response, and assumes to have a greater influence on oral hygiene and systemic problems, unless affected by systemic disease.

In this study, objective investigation has not been performed whether or not subjects are infected with periodontal diseases. For this reason, there is a limitation to verify that adults being detected with oral bacteria have oral diseases. Nevertheless, the authors of this study have identified the fact that adults detected with TM7 are more likely to suffer from acute or chronic gingivitis, and periodontal diseases. Also, we have assumed that there was inflammatory response in the oral cavity, in addition to periodontal disease. More accurate estimation can be made by comparing with the results of oral bacteria detection through further diagnosis.

5. Conclusion

More than 70% of Korean adults are workers, and dental problems predominantly occur in this period. Therefore, examining bacterial species generating oral diseases is crucial for oral health management by identifying the risk factors of oral environment. Furthermore, a marked decrease in bacterial numbers is anticipated through smoking cessation education and habits promoting SFR.

Reference

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<Research Interests>
Biotechnology, Oral bacteria, Oral health