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Relationship Between Oral Malodor and Oral Microbiota

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1. Introduction

Oral malodor, also called halitosis or bad breath, is one of the major complaints made by patients visiting the dentist, ranking behind only dental caries and periodontal disease. It can originate from either systemic or oral conditions, but is usually related to an oral cause. Clinical causes of oral malodor include periodontitis, poor oral hygiene, tongue debris, deep caries, inadequately fitted restorations, endodontic lesions, and low salivary flow [1-5]. Under such conditions, it is thought that either bacterial cell numbers increase or oral bacterial communities shift towards a composition producing high levels of malodor. The major compounds that contribute to oral malodor are volatile sulfur compounds (VSCs), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3) [6, 7]. In addition, methylamine, dimethylamine, propionic acid, butyric acid, indole, scatole, and cadaverine are reported to cause oral malodor. About 90% of the VSCs in mouth air are H_2S and CH_3SH [7], which are produced through bacterial metabolism of sulfur-containing amino acids, such as cysteine and methionine. Gram-negative anaerobes are important producers of VSCs. Persson *et al.* [8] reported that periodontal pathogens isolated from subgingival plaque, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*, generated significant amounts of H_2S and CH_3SH . An examination of the microbiota composition of the tongue biofilm of individuals with no periodontitis, or only a slight degree of periodontitis, suggested that the major species of H_2S -producing bacteria were *Veillonella*, *Actinomyces*, and *Prevotella* [9]. On the other hand, Gram-positive oral bacteria, primarily streptococci, may also promote VSC production by Gram-negative bacteria [10]. Recently, Takeshita *et al.* [11] determined the bacterial composition of saliva, based on terminal-restriction fragment length polymorphism (T-RFLP) profiles using hierarchical cluster analysis, and associated the global composition of indigenous bacterial populations with the severity of oral malodor. The human oral cavity contains more than 500 bacterial species that interact both with each other and host tissues, suggesting that various bacteria may be related to malodor production.

Oral-derived malodor is classified into physiological and pathogenic odor. The microbial composition of the oral cavity varies according to clinical condition; therefore, the most appropriate management strategy may also differ. However, the common goal of regimen for the treatment of oral malodor is the acquisition of a healthy oral condition, including

normal microbiota. In this chapter, we review and summarize previous studies of the relationship between oral malodor and oral bacteria, and discuss oral microbiota-focused therapeutic strategies in addition to mechanical oral hygiene.

2. VSC-producing bacteria and oral malodor

Periodontopathic bacteria

Periodontal disease is the primary cause of pathogenic oral malodor [7, 12]. The majority of the bacterial species isolated from subgingival microbiota produces H_2S and CH_3SH [8]. In particular, bacterial strains forming large amounts of H_2S from L-cysteine were found in the genera *Peptostreptococcus*, *Eubacterium*, *Salenomonas*, *Centipeda*, *Bacteroides*, and *Fusobacterium*, and CH_3SH from L-methionine was formed by some members of the genera *Fusobacterium*, *Bacteroides*, *Porphyromonas*, and *Eubacterium* [8]. Periodontopathic bacteria have been isolated from the tongue coating and saliva, in addition to the periodontal pocket, in subjects with periodontal disease [13, 14]. In particular, the tongue coating is considered a major source of malodor in both periodontally diseased and healthy individuals, because VSC-producing bacteria have easy access to nutrients, such as desquamated epithelium and food debris, in the tongue coating [12, 15, 16]. It is generally observed that patients with chronic periodontitis have far more tongue coating and higher VSC levels than healthy individuals [15, 16]. Tanaka *et al.* [13] quantified five periodontopathic bacteria, including *P. gingivalis*, *T. forsythensis*, *P. intermedia*, *Prevotella nigrescens*, and *T. denticola*, in the tongue debris of patients complaining of halitosis, using real-time polymerase chain reaction (PCR). They found a strong positive correlation between the proportions of periodontopathic bacteria and VSC levels. Furthermore, their data suggest that the proportions of *P. intermedia* and *P. nigrescens* on tongue dorsa are correlated with H_2S concentration. In addition, the proportions of *P. gingivalis* and *P. nigrescens* also showed a strong correlation with CH_3SH concentration. Among the different surfaces in the oral cavity, the bacterial composition of saliva is most similar to that of the tongue coating [17], and bacteria that inhabit other oral surfaces can also be recovered from saliva [18-20]. Therefore, many investigators have used saliva samples to evaluate the relationship between oral malodor and oral microorganisms as reflecting the overall condition of the oral cavity [11, 14, 21]. Awano *et al.* [14] detected the presence of *T. forsythensis*, *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *P. intermedia* by PCR in the saliva of patients complaining of halitosis. They suggested that the presence of *T. forsythensis*, *P. gingivalis*, and *P. intermedia* influenced VSC production, and the presence of *T. forsythensis* in subjects with periodontitis was strongly correlated with VSC concentration in mouth air. One study investigating the relationship between oral malodor and the menstrual cycle reported that VSC, bleeding on probing (BOP), and *P. intermedia* numbers in saliva significantly increased during ovulation in females with periodontitis [21]. In a previous study, we examined the presence of *Helicobacter pylori*, which can cause peptic ulcers and gastric cancer [22, 23] and may cause periodontitis and halitosis [24, 25], in the saliva of patients complaining of halitosis [26]. The presence of *H. pylori* correlated with CH_3SH concentration and periodontal parameters including tooth mobility, periodontal pocket depth, and occult blood in the saliva. Collectively, several periodontopathic bacteria involved in malodor production have been identified and studied.

Normal inhabitants of the tongue

Physiological halitosis in periodontally and systemically healthy people is caused mainly by VSC-producing commensal bacteria that inhabit the tongue coating [27]. According to a study of the topographic distribution of bacterial types on the tongue surface in periodontally healthy subjects, the dorsal posterior to the circumvallate papillae consistently carried the highest load of all bacterial groups [28]. Furthermore, anaerobic, Gram-negative, and VSC-producing (*P. gingivalis*, *F. nucleatum*, and *P. intermedia*) bacterial counts on the dorsal posterior surfaces increased with malodor intensity, whereas aerobic bacterial and *Streptococcus salivarius* counts decreased. An examination of the composition of the microbiota in tongue biofilm suggested that the major species of H₂S-producing bacteria were *Veillonella*, *Actinomyces*, and *Prevotella* in both the odor and no/low odor groups, and the numbers of these bacteria in tongue biofilm were significantly higher in the odor group than in the no/low odor group [9]. The study subjects did not have severe periodontitis (the number of teeth with probing depth ≥ 4 mm was 1.6 and the largest probing depth was 4.0 mm) and therefore these species may contribute to H₂S-production in physiological halitosis.

3. Gram-positive bacteria and oral malodor

VSCs are produced by enzymes that transform S-amino acids into their corresponding sulfides. The proteolytic activity of Gram-negative bacteria is closely associated with this process [29, 30]. Many of the available proteins in the oral cavity are glycoproteins [31], which require removal of their carbohydrate side-chains before the protein core can be degraded [32]. Some researchers have suggested that various glycosidases, such as the β -galactosidase produced by Gram-positive oral bacteria, cleave carbohydrate side-chains of salivary glycoproteins and contribute to VSC-production by Gram-negative oral bacteria [33, 34]. In an *in vitro* study using a model *P. gingivalis*-mediated malodor system, addition of *Streptococcus salivarius* promoted mucin degradation and concomitant malodor production [33]. A clinical study using volunteers reported that the presence of β -galactosidase activity in saliva showed a positive relationship with the organoleptic test score (OLS) of the whole mouth or tongue dorsum [34]. Another study of patients complaining of halitosis reported that the β -galactosidase-positive group showed significantly higher OLS and VSC concentrations as measured by gas chromatography, and tongue-coating scores [35]. Recently, Masuo *et al.* [36] quantified β -galactosidase activity in the saliva of subjects complaining of halitosis, and reported a positive correlation between enzymatic activity in whole saliva and OLS and VSC concentrations in the periodontally healthy group, but not in the periodontitis group. In addition, the plaque index and tongue coating score were positively correlated with β -galactosidase activity in the periodontally healthy group. A bacterial quantitative analysis showed that the numbers of total bacteria, *F. nucleatum*, and *S. salivarius* were positively associated with β -galactosidase activity in the periodontally healthy group. These previous studies suggest that β -galactosidase produced by Gram-positive bacteria plays an important role in physiological oral malodor, although the predominant species are Gram-negative bacteria.

4. Malodor-associated microbiota

As described above, several bacteria involved in malodor production have been identified and studied. The accumulation of investigations regarding VSC-production by specific bacterial species may lead to the discovery of the mechanisms underlying oral malodor. On the other hand, analysis of human oral flora has been limited by conventional culture-dependent methods; thus, a significant proportion of oral bacteria remain uncultured and uncharacterized. Malodor producers are members of the oral microbial ecosystem, which is regulated by numerous interactions among the inhabitants. Recently, a molecular approach based on 16S rRNA was been applied to investigate the diversity of both cultivable and uncultivable species in the human oral cavity [37-39]. Several investigators have attempted to identify the oral bacterial species associated with halitosis by PCR amplification of tongue-debris samples, and cloning and sequencing of 16S rRNA genes [40-42]. Although the size of the study populations was limited (11, 13, and 32, respectively) and their oral condition was not described, the data showed some similarities. The diversity of bacterial species in subjects with halitosis was greater than in those with no halitosis. *Solobacterium moorei* was a key bacterial species identified only in subjects with halitosis.

Despite the lack of fully resolved phylogenetic analyses at the species level, T-RFLP analysis is an effective 16S rRNA-based molecular approach for the rapid assessment and comparison of large numbers of complex bacterial communities [43]. Takeshita *et al.* [11] divided the bacterial composition of the saliva of 240 subjects complaining of halitosis into groups based on T-RFLP using hierarchical cluster analysis. Four types of bacterial community compositions were detected (clusters I, II, III, and IV). Oral malodor parameters, including VSC concentration in mouth air and the OLS, were lower in cluster I. Cluster III exhibited an intermediate pattern both in terms of oral malodor parameters and T-RFLP profile. Bacterial genera corresponding to T-RFs with greater peak area proportions in cluster I were *Streptococcus*, *Granulicatella*, *Rothia*, and *Treponema*. Bacterial candidates representing cluster II, which showed the highest VSC and OLS, were *Veillonella* and *Prevotella*. Bacterial candidates representing cluster IV, which showed the second-highest VSC and OLS, were *Neisseria*, *Haemophilus*, *Aggregatibacter*, *Lautropia*, *Parvimonas*, *Fusobacterium*, and *Porphyromonas*. Two types of bacterial communities (clusters II and IV) were implicated as malodor-associated microbiota. Further studies examining the factors that influence differences in microbial composition are needed.

5. Therapeutic approach to manage oral halitosis from an etiologic standpoint

Basic therapeutic approach

Since malodor originating from the mouth is due to the metabolic degradation of available proteinaceous substrates to malodorous gases by certain oral microorganisms, it can be improved by the reduction of bacterial load and/or nutrient availability, and conversion of VSC to non-volatiles. Prevention and treatment of oral malodor is a primarily a matter of oral hygiene, meaning brushing teeth, flossing, cleaning dentures, and cleaning of the tongue dorsum. Tongue cleaning is considered the most important strategy for the

prevention of oral malodor [44]. In addition, treatment of pathogenic oral malodor requires treatment of oral diseases, such as periodontitis, gingivitis, deep caries, inadequate restorations, endodontic lesions, and dry mouth [45, 46]. Chemical approaches using mouthrinses with antimicrobial properties can reduce oral malodor by reducing the number of microorganisms. The active ingredients in these products are chlorhexidine, essential oils, triclosan, and cetylpyridinium chloride [47]. Other chemical agents can reduce halitosis by chemically neutralizing VSCs. The active ingredients in these products are most commonly zinc and chlorine dioxide [48]. Metal ions with a high affinity for sulfur inhibit the formation of VSCs, which is likely also related to the antibacterial properties of the metal [49, 50]. Chlorine dioxide and the chlorite anion consume amino acids such as cysteine and methionine, which are VSC precursors [51]. In addition, the chlorite anion is powerfully bactericidal [52]. A combination of chemical agents markedly reduced VSC concentrations [53, 54].

Biological therapeutic approach

Biological therapeutic agents, such as lactoferrin and probiotic bacteria, have been employed to control oral malodor [55, 56]. These treatments may involve fewer side effects and are environmentally safe. The expected effects—other than antimicrobial activity—of these agents are diverse and the goal of the treatment is the acquisition of a healthy oral condition, including normal microbiota. Lactoferrin, a member of the transferrin family and a component of milk, saliva, tears, and secondary neutrophil granules, demonstrates immunomodulatory effects and regulates both cell proliferation and iron uptake, in addition to antimicrobial activity [57, 58]. A study using tablets containing bovine lactoferrin and lactoperoxidase in healthy volunteers reported that CH_3SH levels were significantly lower in the test group 10 min after ingestion as compared with the placebo group [56]. No difference in the numbers of salivary bacteria was detected by culturing or quantitative PCR; however, T-RFLP analysis detected one fragment with a significantly lower copy number at 2 h in the test group as compared with the placebo group. Probiotic bacteria, defined as live microorganisms that confer a health benefit on the host when administered in adequate amounts (FAO/WHO 2001), are thought to play a role in the maintenance of oral health [59]. Recently, some studies concerning the effect of probiotic bacteria on maintaining oral health have been reported [60–62]. An open-label pilot trial using tablets containing *Lactobacillus salivarius* WB21 and xylitol reported that VSC levels (H_2S and CH_3SH) and OLS were significantly lower at two weeks in subjects with physiologic halitosis, and OLS and bleeding on probing were significantly lower at four weeks in subjects with oral pathologic halitosis [55]. Quantitative analysis by real-time PCR found that *L. salivarius* and *P. intermedia* numbers were significantly increased in subjects with oral pathologic halitosis at two weeks, but levels of other VSC-producing periodontal bacteria and total bacteria in the saliva were unchanged. Another clinical trial reported that taking a tablet containing *L. salivarius* WB21 reduced the number of *T. forsythensis* in the subgingival plaque of healthy volunteers at four weeks [63]. Thus the effects of biological agents on oral malodor have been confirmed, but no quantitative change in oral bacteria was apparent. Therefore, these effects may be dependent on oral conditions. We also investigated changes in bacterial composition after administration of *L. salivarius* WB21 in subjects with periodontitis. Total bacteria in saliva was significantly reduced in

the test group at two weeks; however, T-RFLP analysis found no difference in bacterial composition between the test and placebo groups (unpublished data). A long-term and large-scale study may be required to clarify the effects of biological agents on oral microbial composition.

6. Conclusions

The present review describes the oral microorganisms and microbiota involved in the production of oral malodor. The oral cavity contains complex, multispecies microbial communities. Residents of these communities display extensive interactions while forming biofilm, carrying out physiological functions, and inducing microbial pathogenesis [64]. The bacterial composition of the oral cavity changes depending on the oral environment; therefore, the bacterial communities associated with oral malodor also differ according to the conditions in the oral cavity. Accumulating information on VSC-producing bacteria, other organisms contributing to the process of oral malodor, and the key species detected in bacterial communities with oral malodor, and comparisons between the bacterial composition of healthy individuals versus those with physiological and pathologic malodor will serve as a basis for exogenously modulating the interactions between biofilm constituents, thus resulting in novel approaches for controlling biofilm activities. In the future, oral malodor therapy approaches should be modified from “remove and kill all” to “acquire healthy oral microbiota.”

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Geriatric dentistry, or gerodontics, is the branch of dental care dealing with older adults involving the diagnosis, prevention, and treatment of problems associated with normal aging and age-related diseases as part of an interdisciplinary team with other healthcare professionals. Prosthodontics is the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/or oral and maxillofacial tissues using biocompatible materials. Periodontology, or Periodontics, is the specialty of oral healthcare that concerns supporting structures of teeth, diseases, and conditions that affect them. The supporting tissues are known as the periodontium, which includes the gingiva (gums), alveolar bone, cementum, and the periodontal ligament. Oral biology deals with the microbiota and their interaction within the oral region. Research in oral health and systemic conditions concerns the effect of various systemic conditions on the oral cavity and conversely helps to diagnose various systemic conditions.

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