Tongue coating
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CHAPTER 1

General Introduction

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CHAPTER 1

Introduction
The tongue
The human tongue is a muscular organ situated in the floor of the mouth, covered with a mucous membrane. Its function is associated with chewing, swallowing, vocalization, and taste. Anatomically, the tongue can be distinguished into root, tip, and dorsal and ventral surfaces. The V-shaped sulcus terminalis on the dorsal surface separates the oral and pharyngeal parts of the tongue. The tongue surface consists of tiny protrusions called tongue papillae, which can be classified into four types: filiform, fungiform, circumvallate, and foliate papillae [1]. Filiform papillae, present at the anterior part of the tongue, have a core length of 0.5 mm with a central crater and uplifted borders. They are responsible for tongue anterior roughness. The foliate papillae are located at the edges, whereas the vallate papillae are located at the posterior tongue with 1 mm height and 2–3 mm diameter [2]. Fungiform papillae have a core length of about 0.5–0.8 mm. Fungiform, foliate, and circumvallate papillae contain the sensory cells involved in taste sensation [40].

Traditionally, visible aspects of the tongue have played an important role in oral and general health. The tongue is involved in various diseases such as vascular and lymphatic lesions (infantile hemangiomatous and oral varices), reactive and inflammatory processes (hairy tongue, pigmented fungiform papillae of the tongue, benign migratory glossitis, and fissured tongue), infections (oral hairy leukoplakia, herpes simplex and varicella zoster virus infections, human papillomavirus, and candidiasis), premalignant lesions (leukoplakia and erythroplakia), malignant lesions (squamous cell carcinoma, Kaposi sarcoma, and lymphatic proliferative diseases), and signs of systemic conditions such as nutritional deficiency and systemic amyloidosis [3]. The tongue can reflect symptoms of systemic diseases and serves as a diagnostic indicator and for that reason is often called “a true mirror of the body.” For example, in HIV, the tongue is associated with specific symptoms, including a white patch with a corrugated or hairy appearance on the lateral tongue margin that represents hairy leukoplakia, which helps in early diagnosis of the infection [4]. Unlike other parts of the oral cavity, the patient can inspect the changes of the tongue easily and can misinterpret changes that they perceive. The involvement of the tongue in various disorders and diseases poses a diagnostic and therapeutic challenge to a general dental practitioner.

The tongue coating
In general, the coating is a characteristic feature of the tongue, comprising a thin white coat on the dorsal (middle and posterior) surface in healthy individuals [5]. The components of tongue coating are divided into i) cellular and ii) non-cellular. Cellular components are desquamated keratinized epithelial cells, bacteria and white cell
exudates. The non-cellular components contain proteins from saliva along with food debris and postnasal and gingival secretions [6].

**Formation of the tongue coating**
The formation of the tongue coating is thought to relate to proliferation and apoptosis of glossal epithelial cells [7]. A microscopic study on tongue ultrastructure indicated that the rate of multiplication of epithelial cells, quantity of desmosomes, and membrane-coating granules are responsible for tongue coating formation [8]. In addition, filiform papillae have a role in coating formation, based on the ultra-structural observation of tongue coating using transmission electron microscopy, which showed bacteria along with desquamated epithelial cells originating from the filiform papillae [9].

**The medical significance of the tongue coating**
In traditional Chinese medicine (TCM), the tongue and its coating play a vital role in disease diagnosis. According to TCM, the features of tongue coating such as color, thickness, and distribution represent the strength and depth of pathogenic factors [10]. According to TCM, the tongue is connected with other parts of the body such as heart, lung, liver, kidney, spleen, gall bladder, intestine (large and small), and stomach [10]. A change in the function of any of these organs is then reflected in the coating morphology and thus considered as an incidental symptom in Chinese medicine [11]. In ancient Greek medicine, tongue coating features could be a major symptom of acute, transient, or superficial conditions. In early (1828) western medicine, the amount of tongue coating and its color were considered in diagnosis of diseases like hepatitis and pneumonia and their progression [12]. Table 1 shows how tongue coating has been used in diagnosis. However, because of today’s advanced and sophisticated techniques, tongue coating–based diagnosis receives less attention.

<table>
<thead>
<tr>
<th>Tongue coating</th>
<th>Diseases</th>
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<tbody>
<tr>
<td>A coating on one side</td>
<td>Lung infection on the corresponding side</td>
</tr>
<tr>
<td>Tongue with a lard-like coating</td>
<td>Slow recovery in patients with fever</td>
</tr>
<tr>
<td>Yellow coating</td>
<td>Indicative of hepatitis and pneumonia</td>
</tr>
<tr>
<td>Yellow coating on one side</td>
<td>Hemiplegia or migraine</td>
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</tbody>
</table>

*Table 1: Tongue coating in diagnosis of various diseases*

**Examination of the tongue coating**
Different features are used to describe the tongue coating and include thickness, area covered on the tongue surface, and discoloration. Traditionally, tongue coating is first
evaluated by visual examination from tip to root. The thickness of the coating is conveyed as none, thin (tongue body is visible), and thick (tongue body is not visible) [6]. These rather subjective criteria are not sufficient to accurately and reproducibly describe the amount of tongue coating, which has led to high inter- and intra-practitioner variation and underscores the need for a standard method [6]. In recent years, several authors have proposed tongue coating indices (e.g., Miyazaki et al. [13], Mantilla Gomez [5], Oho et al. [14], and Winkel et al. [15]). The Winkel Tongue Coating Index is generally considered a feasible method because of the simple evaluation criteria and easy interpretation of the scores [16].

Tongue coating in intra-oral halitosis
Halitosis is defined as a foul odor emanating from the mouth and can be classified into four types based on origin: transient or temporary halitosis, extra-oral halitosis, intra-oral halitosis, and pseudohalitosis (Fig. 1). Transient halitosis is temporary and caused by certain food intake (e.g., garlic) and morning bad breath that will disappear after breakfast and oral hygiene practices [17, 18]. Extra-oral halitosis (EOH) originates from a pathological condition outside the oral cavity and is categorized into blood-borne and non–blood-borne causes [18]. Non–blood-borne halitosis is caused by disorders in the nasal, paranasal, and laryngeal regions and the pulmonary or upper digestive tract. Blood-borne causes of EOH involve genetic causes [43], kidney and liver diseases, diabetes, metabolic disorders and certain certain drugs [18]. In case of blood-borne causes, gaseous compounds that originate from disorders in the body (e.g., hepatic cirrhosis) are exhaled via the lungs and the oral cavity [18].

Intra-oral halitosis (IOH) arises from the oral cavity and is responsible for 90% of halitosis cases. The consequences of IOH are often underestimated and can involve considerable impact on an individual’s social behavior and psyche. The current prevalence of IOH is not clear, but recent studies suggest a prevalence of 22% to 50% worldwide [21]. A survey carried out in the Netherlands in 2005 revealed that halitosis is among the top 100 greatest human health issues (TNS-NIPO) [19]. The physiological cause of IOH is strongly associated with tongue coating, whereas the pathological cause of IOH is gingivitis/periodontitis. These associations were demonstrated in a study involving 2000 patients in which a tongue coating was the cause for 51% of IOH, gingivitis/periodontitis was responsible for only 13%, and the combination was responsible for 22% of the cases. Other risk factors include stress and xerostomia [20].

In people with IOH, the amount of tongue coating is relatively high compared to those without it [22]. In daily life, a tongue coating is very thin because of the routine tongue movements during chewing and swallowing. Additionally, saliva
production and dietary elements (e.g., fibrous foods) are strongly involved in cleansing the tongue. Factors that increase the amount of tongue coating are age and age-related. Elderly people often struggle to cope with oral hygiene and have an increased intake of soft food, with a reduced natural cleansing of the tongue by saliva and tongue movements. Among the several factors, oral hygiene has the strongest impact in the formation of coating. The factors outside the oral cavity such as gastrointestinal and liver diseases may also contribute to the thickness of the coating [23].

**Tongue coating and volatile sulfur compounds**
The tongue coating is believed to be the major source of volatile sulfur compounds (VSCs), which are key elements in IOH [24] [25]. VSCs are produced by anaerobic bacteria of the tongue dorsum that degrade sulfur-containing amino acids to VSCs [26]. The most common VSCs are hydrogen sulfide ($\text{H}_2\text{S}$), methyl mercaptan ($\text{CH}_3\text{SH}$), and dimethyl sulfide ($\text{CH}_3\text{S}\text{CH}_3$) [27]. $\text{H}_2\text{S}$ and $\text{CH}_3\text{SH}$ together constitute
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approximately 90% of VSCs in IOH. The important organic substrates for VSC production are cysteine and methionine from the saliva, gingival, and crevicular fluid and the tongue surface. In addition, bacterial degradation of diamines (tryptophan) produces indole and skatole, whereas degradation of polyamines (lysine and ornithine) can result in cadaverin and putresin [19]. The contribution of indole, skatole, cadaverin, and putresin to IOH is controversial [28].

The microbiome and the tongue coating

Several studies have demonstrated the relation between the tongue coating microbiome and IOH. An in vitro study showed that the production of oral malodor was associated with a shift in the microflora from Gram-positive to Gram-negative anaerobes [29]. Another in vitro analysis showed that certain oral bacterial species, when incubated with serum containing L-cysteine and L-methionine, produced mainly H$_2$S and CH$_3$SH [30]. Because the tongue dorsum has a surface area of 25 cm$^2$ [36] with irregular topography from the presence of numerous fissures, crypts, and papillae, it provides shelter for the tongue microbiome. Conventional anaerobic culture studies of IOH yielded isolated Peptostreptococcus anaerobius, Collinsella aerofaciens, Eubacterium group, Actinomyces spp., Eikenella corrodens, Veillonella spp., Fusobacterium nucleatum, pigmented Prevotella spp., Selenomonas spp., Actinomyces turicensis, Collinsella aerofaciens, Eubacterium saburreum, Eubacterium timidum, Prevotella tannerae, Campylobacter concisus, Campylobacter mucosalis, Leptotrichia buccalis, Selenomonas flueggei, and Centipeda periodontii [31]. The anaerobic culture method had limitations in the cultivation of existent microbes, so the advent of sequencing-based technology has revolutionized medical research.

In culture-independent techniques, 16S rRNA sequencing of cloned genes from IOH samples showed the presence of Atopobium parvulum, Dialister spp., Eubacterium sulci, a phylogtype of the uncultivated phylum TM7, Solobacterium moorei, and a phylogtype of Streptococcus, all highly associated with IOH [22]. Another study using 16S rRNA sequencing of tongue samples from patients with IOH showed the presence of Lysobacter-type species, S. salivarius, Prevotella melaninogenica, Prevotella veroralis, and Prevotella pallens. These authors concluded that the tongue microbiome in IOH has great species diversity and is complex [32]. The combination of anaerobic culture technique and amplification of 16S ribosomal DNA has revealed the presence of 32 species, including 13 non-cultivable species in halitosis patients [33].

Of interest, S. moorei was given more importance in several studies because of its presence in people with IOH compared to those without it. One such study revealed the presence of S. moorei only in participants with halitosis but not in any of
the control participants [33]. In a later study involving S. moorei, clinical factors of IOH (such as H2S, CH3SH, (CH3)2S, and total VSCs) and tongue coating indices showed a significant correlation with each other [34]. However, quantitative polymerase chain reaction on tongue samples of IOH showed only a slight elevation of S. moorei in patients with IOH (98.8%) compared to controls (88.9%) [34]. The study showing that S. moorei produces VSCs also showed that S. moorei could produce VSCs from serum only in the presence of exogenous proteases such as pancreatic trypsin or Porphyromonas gingivalis gingipains [35]. These studies did not give further information on the crosstalk between the specific bacteria and IOH.

A study on the bacterial load of the tongue dorsum in participants with IOH showed a significant correlation between total bacterial load and IOH [26], but another study showed the opposite [37]. The authors of this latter study hypothesized that the tongue coating per se and not the bacteria was responsible for IOH [37]. Furthermore, the determination of the bacterial load before and after the tongue cleaning intervention showed no reduction in bacterial load [2]. The increase in certain bacterial species rather than the bacterial load was believed to be the responsible factor in the IOH. Based on this concept, a study showed an increase in H2S-producing bacteria in participants with IOH. Thus, IOH is probably caused by bacteria of the oral cavity, and an increase in their number plays a role in IOH [38].

**Intra-oral conditions affecting the tongue microbiome in IOH**

The oral microbiome might change in composition, but we do not understand which factors initiate these changes. Altered physical parameters (environmental factors) such as pH, oxidation–reduction potential, or a low carbohydrate environment might play a role [29]. Previous studies on IOH focused more on microbial aspects rather than environmental factors. In vitro studies on microbes have demonstrated their ability to produce VSCs but did not reveal bacterial physiological changes. The cause of IOH is complex and may involve increased bacterial numbers along with metabolite-related factors [17]. Thus, a deeper understanding of bacterial flora dynamics and metabolites is required for developing an effective treatment strategy for IOH.

**Treatment for tongue coating–related IOH**

Treatment for IOH first involves VSC reduction. Tooth brushing, interdental cleaning, and tongue scraping, which reduce the total oral bacterial load including VSC-producing bacteria, can achieve this. These steps also reduce residual food matter and cellular debris from the gingiva and tongue coating. Mechanical approaches can reduce oral malodor, but their efficiency in treating chronic oral malodor is limited. Chemical treatment includes the use of mouth rinses. Effective mouth rinses contain
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antibacterial agents that affect bacterial growth and neutralize bacterial VSCs. In-depth investigations into the mechanism of IOH formation is required to improve treatment of IOH effectively [39].

Tongue cleaning effect on taste perception
The primary sensory units of the taste system, the taste buds, are located in the taste papillae covering the tongue surface. Taste buds are embedded under the keratinous layer of the papillae with a taste pore exposed to the external milieu [40]. The five basic taste qualities are sweet, salty, sour, bitter, and umami. Among these qualities, sweet and salty tastes have a great impact on health-related problems. In particular, salt is a strong risk factor for hypertension, stroke, and obesity. According to the World Health Organization, human salt intake is high, ranging from 5–9 g/day, and the agency recommends reduction of salt intake [41]. The tongue-cleaning oral hygiene regimen significantly improves the salt taste sensation and might help to reduce salt intake [2, 42].

Aim of this thesis
In individuals with a healthy periodontium, tongue coating is the predominant cause for IOH, and anaerobic bacteria on the tongue dorsum produce VSCs that contribute to IOH. An improved understanding of the tongue microbiome will ultimately permit expansion of therapeutic strategies and is the aim of this thesis. Hence, the following research questions were formulated.

1. What is the impact of tongue coating in IOH, and what causes tongue coating formation?
2. What is the microbial composition of the tongue coating in IOH, and does the tongue microbiome induce coating formation?
3. Is IOH a metabolite-related condition, is microbiome function metabolite-related, and how are they related to VSC production?
4. Does the removal of tongue coating increase taste perception?

Outline of the thesis
In chapter 1, a topical review is given on tongue coating, based on current knowledge, and its association with IOH. Also, the impact of tongue coating on general health is discussed.

In chapter 2, the tongue microbial composition in people with and without IOH is discussed, based on using 16S amplicon Illumina sequencing, an advanced sequencing technology, to study the microbiome.
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In chapter 3, the formation of the tongue coating and the metabolic pathway involved in formation of VSCs is investigated using a non-targeted metabolomics approach, liquid chromatography–tandem mass spectrometry.

In chapter 4, the effect of removal of the coating by tongue cleaning and its relation to the intensity of salty taste perception is investigated.

In chapter 5, the formation of the tongue coating, the microbiome, and its metabolites in relation to IOH are summarized.

References
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GENERAL INTRODUCTION
