EFFECT OF EPIGALLOCATECHIN-3-GALLATE IN GREEN TEA ON MALODOR: A CLINICAL STUDY

Mr. Pisal Sampatanukul

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for the Degree of Master of Science Program in Periodontics

Department of Periodontology

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ผลของอีพิแกลโลคาเทชิน-3-แกลเลทในชาเขียวต่อกลิ่นปาก: การศึกษาในคลินิก

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By	Mr. Pisal Sampatanukul
Field of Study	Periodontics
Thesis Advisor	Assistant Professor Suphot Tamsailom
Thesis Co-advisor	Associate Professor Em-on Benjavongkulchai, Ph.D.

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……Dean of the Faculty of Dentistry
(Assistant Professor Thitima Pusiri)

THESIS COMMITTEE

……Chairman
(Assistant Professor Ornanong Vanichjakvong)

……Thesis Advisor
(Assistant Professor Suphot Tamsailom)

……Thesis Co-advisor
(Associate Professor Em-on Benjavongkulchai)

……Member
(Associate Professor Nualchavee Hongprasong)

……Member
(Dr. Thongchai Vachirarojpisan)

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การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบผลของอีพิแกลโลคาเทชิน-3-แกลเลทจากชาเขียวต่อก๊าซที่มีซัลเฟอร์เป็นองค์ประกอบของการมีกลิ่นปาก อาสาสมัครที่เป็นโรคปริทันต์อักเสบและมีค่าก๊าซที่มีซัลเฟอร์เป็นองค์ประกอบในช่องปากตั้งแต่ 125 ส่วนในพันล้านส่วน จำนวน 30 คน ได้รับการสุ่มให้อมน้ำยาบ้วนปากด้วยสารทดลอง (อีพิแกลโลคาเทชิน-3-แกลเลตความเข้มข้น 50 มก/100 มล ในน้ำกลั่น) หรือสารควบคุม (น้ำกลั่น) เป็นเวลา 2 นาที หลังจากนั้นจะสลับการให้ใช้น้ำยาบ้วนปากอีกตัวหนึ่งโดยมีระยะพักระหว่างกลุ่มการทดลองอย่างน้อย 1 สัปดาห์ ซึ่งทั้งผู้ทำการวิจัยและอาสาสมัครจะไม่ทราบชนิดของน้ำยาบ้วนปาก การวัดค่าก๊าซที่มีซัลเฟอร์เป็นองค์ประกอบซึ่งได้แก่ ไฮโดรเจนซัลไฟด์ เมธิลเมอร์แคปแทน และไดเมธิลซัลไฟด์ ทำโดยใช้เครื่องวัดก๊าซโครมาโตกราฟฟีอย่างง่าย (Oral Chroma®) ที่จุดเริ่มต้น และที่ 15 30 60 และ 120 นาที หลังการอมน้ำยาบ้วนปาก ทำการเปรียบเทียบค่าแตกต่างของก๊าซที่เกิดขึ้นหลังการอมน้ำยาบ้วนปากเทียบกับจุดเริ่มต้นระหว่างกลุ่มทดลองและกลุ่มควบคุมโดยใช้สถิติชนิดสติวเดนท์ทีเทสท์หรือวิลคอกสันไซแรงค์เทสท์ ผลปรากฏว่าอีพิแกลโลคาเทชิน-3-แกลเลทมีผลการลดก๊าซเมธิลเมอร์แคปแทนได้เหนือกว่ากลุ่มควบคุม ที่ 30 60 และ 120 นาที หลังการอมน้ำยาบ้วนปากอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ในขณะที่ผลการลดก๊าซไฮโดรเจนซัลไฟด์ และไดเมธิลซัลไฟด์ ไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) การวิจัยนี้สรุปว่า อีพิแกลโลคาเทชิน-3-แกลเลท มีผลในการลดก๊าซเมธิลเมอร์แคปแทน ซึ่งเป็นก๊าซที่พบเป็นหลักในกลิ่นปากของผู้ป่วยโรคปริทันต์อักเสบ ทั้งนี้อาจเป็นผลจากฤทธิ์ในการต้านจุลชีพของอีพิแกลโลคาเทชิน-3-แกลเลทต่อเชื้อก่อโรคปริทันต์ซึ่งเป็นเชื้อหลักในการกำเนิดก๊าซดังกล่าวในช่องปาก

ภาควิชาปริทันตวิทยา	ลายมือชื่อนิสิต.....……
สาขาวิชาปริทันตศาสตร์	ลายมือชื่ออาจารย์ที่ปรึกษา.....……
ปีการศึกษา 2550	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....……

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KEY WORD : EPIGALLOCATECHIN-3-GALLATE / VOLATILE SULPHUR COMPOUND / MALODOR / GREEN TEA

PISAL SAMPATANUKUL : EFFECT OF EPIGALLOCATECHIN-3-GALLATE IN GREEN TEA ON MALODOR: A CLINICAL STUDY.

THESIS ADVISOR: ASST. PROF. SUPHOT TAMSAILOM THESIS CO-ADVISOR: ASSOC. PROF. EM-ON BENJAVONGKULCHAI, Ph.D., 58 pp.

The purpose of this study was to investigate the effect of epigallocatechin-3-gallate from green tea on oral volatile sulphur compounds (VSCs) in malodor. Thirty periodontitis subjects with baseline VSCs $\geq$ 125 ppb were randomly assigned to use the test mouthrinse (50 mg/100 ml EGCG in distilled water) and control agent (distilled water) for 2 min in the double-blinded crossover study with at least 1 week washout period. Levels of hydrogen sulphide, methyl mercaptan and dimethyl sulphide in both test and control groups were measured by semiconductor simplified gas chromatography (Oral Chroma®) at baseline and 15, 30, 60 and 120 min after mouth rinsing. The gas changes were compared between the control and EGCG mouthrinse using Student's t-test or Wilcoxon signed rank test. The results demonstrated that EGCG had significantly superior ability in gas reduction for methyl mercaptan than the control after mouth rinsing at 30, 60 and 120 min ($P<0.05$), while showed no significant gas reduction for hydrogen sulphide and dimethyl sulphide ($P>0.05$). This study concluded that EGCG had beneficial effect in reducing methyl mercaptan, which is the major component of VSC in periodontitis patient. It may be due to the antimicrobial activity of EGCG upon periodontal pathogens which are the prominent methyl mercaptan producers in oral cavity.

Department of Periodontology
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Student's signature ……..
Advisor's signature……..
Co-Advisor's signature……

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CHAPTER I

INTRODUCTION

Background and rationales

Halitosis or oral malodor is the foul or offensive odor emanating from the oral cavity. Halitosis can be divided into many categories depending mainly on the etiology from which it comes. However, Delanghe et al. (1999) showed that the majority of halitosis cases come from the oral cavity which is called the true halitosis. It directly links to the formation of volatile sulphur compounds (VSCs) including hydrogen sulphide, methyl mercaptan and dimethyl sulphide which are the degradation products of sulphur containing amino acids such as methionine, cysteine and cystine from the bacteria (Tonzetich and Carpenter, 1971; Persson et al., 1990). It was found that Gram negative bacteria especially those periodontal associated bacteria eg. *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* are the main VSC producers (McNamara, Alexander, and Lee, 1972; Loesche, 1999).

The bacteria which are the VSC producers inhabit in different area in the oral cavity. The common habitats of malodor associated bacteria are the posterior dorsum of tongue which strongly correlates with malodor (Coli and Tonzetich, 1992; Bosy et al., 1994) and the periodontal pockets (McNamara et al., 1972). Tongue especially the area of fissure and crypt can harbor large amount of Gram negative bacteria (De Boever and Loesche, 1995). Moreover, it has been proven that degree of tongue coating plays significant role in the breath odor formation (Yaegaki and Sanada, 1992; Bosy et al. 1994; De Boever and Loesche, 1995), even though removal of tongue coating seems to have little effect on bacterial load (Quirynen, Teughels, and van Steenberghe, 2003). Additionally, it is logical to assume a positive correlation between VSC levels in the mouth and periodontal disease which is indicated by the extent of periodontal pocket depth and gingival bleeding tendency (Coli and Tonzetich, 1992; Yaegaki and Sanada,

1992a). Therefore, to inhibit malodor, tongue and periodontal pocket are crucial areas to be focused on.

Since it has been established that reducing plaque formation is the best way to fight periodontal pathogens and also malodor, many anti-plaque and antimicrobial agents have been introduced. Natural plants are a good source of anti-microbial agents eg. Papua-mace extract, flavono flavor, raspberry extract, etc. (Saeki et al., 1993). Green tea, which is the most popular beverage, is also one of them. It contains catechins which are compounds containing polyphenol therefore it can act as anti-oxidant polyphenol compounds. There are 4 catechins in tea ie. epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG). EGCG has been reported to have several attractive benefits to human beings e.g. anti-carcinogenic ability, antimicrobial and antioxidative effect to some substances such as reactive oxygen substances (ROS) from neutrophils, etc. Okamoto and colleagues (2003) found the negative effect of catechins on *Prevotella intermedia* whereas several bacteria such as *Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Prevotella denticola* and *Streptococcus mutans* can be inhibited by catechins at different MICs (Saeki et al., 1993). Besides, EGCG can inhibit cysteine proteinase activity, growth and also epithelial adherence *of Porphyromonas gingivalis* (Sakanaka et al., 1996).

Therefore, it is reasonable that EGCG may have some negative effects on VSCs formation which is the main cause of malodor. However, this effect has not been clearly established so the objective of this study is to investigate the effect of EGCG on VSC concentration in mild to severe periodontitis which are prone to have striking malodor.

Objectives

To investigate whether EGCG can reduce any components of three major oral VSCs in periodontitis patients.

Scope

This experimental study is performed in human subject with periodontitis. Each subject had at least 125 ppb of VSC level.

Assumption

This study used EGCG as the representative of the catechin polyphenol compounds because it is the most widely studied, most effective antimicrobial agent and also the major catechin in green tea.

Limitation

There is only one concentration of EGCG used in this study.

Operational definitions

Clinical attachment loss is the loss of attachment measured by the UNC-15 periodontal probe using the cemento-enamel junction (CEJ) as the reference point. Periodontitis is the periodontal attachment loss at least 1 mm from CEJ.

Expected benefits

If this study suggests that EGCG can reduce malodor, it may be used to treat malodor patients eg. in form of mouthrinse.

Research question

Can epigallocatechin-3-gallate bring about reduction of any type of oral VSCs in periodontitis patients?

Hypothesis

1. H_0: There are no statistical differences between hydrogen sulphide level in the control and that in the test group.

H_A: There are statistical differences between hydrogen sulphide level in the control and that in the test group.

2. H_0: There are no statistical differences between methyl mercaptan level in the control and that in the test group.

H_A: There are statistical differences between methyl mercaptan level in the control and that in the test group.

3. H_0: There are no statistical differences between dimethyl sulphide level in the control and that in the test group.

H_A: There are statistical differences between dimethyl sulphide level in the control and that in the test group.

CHAPTER II

LITERATURE REVIEW

Malodor

Most of the malodor are physiological or transient condition which is not difficult to treat. However, ten to thirty percent of malodor were really chronic conditions for which treatment have to be sought. Malodor may bring about low self-esteem, low confidence, psychological stress and even social isolation. Oral malodor has several etiologies. It can be classified into the following categories.

1. True halitosis

   True halitosis is oral malodor beyond socially acceptable level and is divided into physiological and pathological halitosis. Both type of true halitosis can also take place concurrently. Pathological halitosis is categorized into extraoral and intraoral.

2. Pseudohalitosis

   Pseudohalitosis is the term used to describe a condition in which a patient believes that significant malodor is present but examination reveals no offensive odor (Yaegaki and Coil, 2000, 1999).

3. Halitophobia

   Halitophobia is characterized by a patient's persistent believe that one has halitosis despite the treatment and counselling (Yaegaki and Coil, 1999).

In many cases the problems come from the oral cavity as intraoral pathologic halitosis in the presence of oral disease, pathologic conditions, xerostomia or periodontal disease while physiologic halitosis results from the putrefaction mostly in the dorsum of tongue or digestive process in the stomach. Offensive odors emanating from the oral cavity can also come from certain odoriferous foods, such as garlic, onion, some medications (Lu, 1982), foreign bodies (Finkelstein et al., 1993), systemic

illnesses (Preti et al., 1992) and various infections (Murata et al., 2002). However they are most generally caused by bacteria (McNamara et al., 1972). Many non-oral sites have been related to oral malodor, including the air routes (nose, sinuses, pharynx and lungs), the gastrointestinal tract, kidneys and liver (Preti et al., 1992). The conditions that favour the retention of anaerobic, mainly Gram-negative, bacteria will predispose the development of bad breath. Besides periodontal pockets, the most important retention site is the dorsum of the tongue with a large number of papillae. During the night and the period between meals, there are conditions that are optimal for odor production. Some systemic diseases, such as diabetes mellitus, uremia and hepatic disease, induce metabolic products that are detectable as oral smells. It seems to be easy to recognize oral malodor, but identifying the exact cause is more difficult. The clinical detection and interpretation of oral malodor bring about the diagnosis and treatment of underlying disease.

The incidence of 15-20% malodor increases with age and malodor seems to occur more in male than female. The malodor is, in fact, the present of the volatile sulphur compound (VSCs) in the mouth. Normally, there is the baseline of volatile sulphur compound level in the oral cavity which does not cause malodor or halitosis. However, in some people, volatile sulphur compound level is so excessive that it can be perceived by other people. The threshold limit of volatile sulphur compound that can be recognized by other people is 125 ppb. However, the major VSCs that really cause halitosis are hydrogen sulphide, methyl mercaptan, and dimethyl sulphide. These gas level will change with time during the day in the so called “daily circardian rhythm of malodor” pattern. The peak value of gases will be approached after night sleep, before lunch and before dinner. Immediately after meal, the volatile sulphur compound will be reduced due to the more acidic pH, reduction of VSC-producing bacteria and increment of salivary flow that interfere with the VSCs mainly produced from the anaerobic bacteria. Such bacteria can use the sulphur-containing amino acid e.g. methionine, cysteine or cystine as the substrate and cause the VSCs as their by-products. The

example of these bacteria are *Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum and Prevotella nigrescens* and all of them can be found in the patients with periodontitis. So these patients seem to have malodor more frequently than persons who are periodontally healthy. Among these three major VSCs, hydrogen sulphide and methyl mercaptan are predominant in mouth air. Both compounds are highly toxic, especially methyl mercaptan (Scully et al., 1997). VSCs can increase the permeability of the oral mucosa (Ng and Tonzetich, 1984) and decrease protein or collagen synthesis (Johnson, Ng and Tonzetich, 1992; Johnson, Yaegaki and Tonzetich, 1996). It is possible that methyl mercaptan within a periodontal pocket may involve in the induction or progression of periodontal disease. These findings indicate the close relationship between anaerobic bacteria, periodontitis and malodor.

Hydrogen sulphide

Hydrogen sulphide is one of the most important gas components in the oral malodor. With the molecular formula of H_2S (Figure 1), it is the colorless, rotten egg odor and flammable gas. It commonly comes from the bacterial breakdown of sulphur-containing organic matter in the absence of oxygen (anaerobic condition). It can be found in natural gas and some well waters. It differs from elemental sulfur which has no odor.

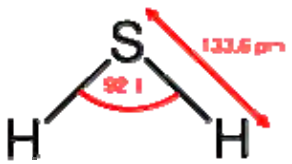


Figure 1: Molecular structure of H_2S

Hydrogen sulphide is weakly acidic, dissociating in aqueous solution into hydrogen cations (H^+) and the hydro sulphide anion (HS^-). Hydrogen sulphide in colon may cause

ulcerative colitis. Normally hydrogen sulphide can come from bacteria. Sulphate-reducing bacteria obtain energy by oxidizing organic matter or hydrogen with sulphate, producing hydrogen sulphide. These bacteria are prevalent in low oxygen condition. *Salmonella* as sulphur-reducing bacterium has the ability to produce hydrogen sulphide. *Streptomycetes* are also found to produce hydrogen sulphide (Kuster and Williams, 1964). During the digestion of sulphur-containing amino acids, anaerobic bacteria can also liberate hydrogen sulphide. Such bacterial action may contribute to bad breath. In 24 h, all strain of *Bacteroides paratyphosus B* produce hydrogen sulphide *in vitro* as well as *Bacteroides typhosus*. Glucose and lactose have little effect upon hydrogen sulphide formation.

For intraoral environment, several bacterial species form significant amounts of hydrogen sulphide from L-cysteine. The active bacteria are *Peptostreptococcus, Eubacterium, Selenomonas, Centipeda, Bacteroides and Fusobacterium.* The most potent producers of hydrogen sulphide are *Treponema denticola* and the black-pigmented species i.e. *Bacteroides intermedius, Bacteroides loescheii, Porphyromonas endodontalis* and *Porphyromonas gingivalis* (van Winkelhoff et al., 1986).

Methyl mercaptan

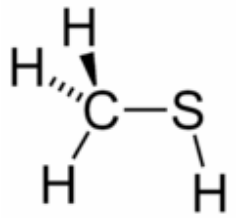


Figure 2: Molecular structure of CH_3SH

Methyl mercaptan also known as methanethiol or mercaptomethane is a colorless gas with a smell like rotten cabbage. It is natural substance found in blood and tissue of human, animals and in certain foods, for example nuts and cheese. With the molecular formula of CH_3SH (Figure 2), it is one of the major odorous gases in the oral cavity.

Methyl mercaptan can be produced by oral bacteria e.g. *Fusobacterium sp, Bacteroides sp, Porphyromonas sp and Eubacterium sp*. *Porphyromonas endodontalis* and *Porphyromonas gingivalis* produce significant amounts of methyl mercaptan in serum (Persson et al., 1990). Yoshimura et al. (2000) studied the formation of methyl mercaptan from L-methionine. To examine the role of methyl mercaptan in the pathogenesis of *Porphyromonas gingivalis*, the L-methionine-α-deamino-γ-mercaptomethane-lyase (METase)-deficient mutant of *Porphyromonas gingivalis* W83 was constructed and found that the invasive strains W83 and W50 produced large amounts of methyl mercaptan. Methyl mercaptan not only is one of the sources of oral malodor, but may also play a role in the pathogenicity of *Porphyromonas gingivalis* in host tissue destruction. Coil and Tonzetich (1992) reported that the increase in the ratio of methyl mercaptan to hydrogen sulphide in human gingival crevicular sites was correlated with deeper pockets or bleeding pockets. Exposure to methyl mercaptan alters protein synthesis in human gingival fibroblasts (Johnson, Ng, and Tonzetich, 1992) and inhibits the migration of periodontal ligament cells (Lancero, Niu, and Johnson, 1992). Methyl mercaptan is produced from L-methionine by the enzymatic action of METase, which catalyzes the α, γ elimination of L-methionine to produce α-ketobutyrate, methyl mercaptan and ammonia. This enzyme was detected in anaerobic, non-oral microorganisms, such as *Pseudomonas*, *Trichomonas* and *Clostridium* (Kreis and Hession, 1973; Mckie et al., 1998). In addition, *Porphyromonas gingivalis*, a black-pigmented anaerobe, which is implicated as a major pathogen in adult periodontitis, is known to produce large amounts of methyl mercaptan in human serum. Among *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Estericia coli* and two strains of *Porphyromonas gingivalis*, *Porphyromonas gingivalis* strain W83 produces the highest amount of methyl mercaptan. This strain is also known as an invasive virulent strain (Neiders et al., 1989). Therefore, it is possible that methyl mercaptan may be one of the virulence factors of *Porphyromonas gingivalis* especially strain W83.

From the knowledge obtained above, these finding suggest that methyl mercaptan may not only responsible for oral malodor but also contributes to the pathogenesis of periodontal disease.

Dimethyl sulphide

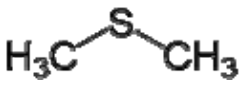


Figure 3: Molecular structure of $(CH_3)_2S$

Dimethyl sulphide, also known as methylthiomethane, is a sulphur-containing organic chemical compound with the molecular formula of $(CH_3)_2S$ (Figure 3). In a vapor form, it comes from cooking of some vegetables such as corn and cabbage. Moreover, it can be found in seafood and can come from bacterial metabolism of methanethiol. This gas is normally found in low level in oral cavity.

Measurement of malodor

Since the oral malodor is the major concern of many people, there have been many attempts to find out the appropriate method to accurately measure the level of malodor. The conventional method which is still acceptable is the organoleptic measurement. However, it depends on the judgement of the trained investigator to subjectively make decision on the level of malodor. The investigator will keep the distance of about 10 cm away from the subject and then inhale the blowing of the subject directly so that the investigater can record the subjective level of the malodor by “organoleptic scale” (Rosenberg et al., 1991). Although this method is easy and can be used conveniently in large number of subjects, its subjective manner seems not to be completely reliable in many situations.

Consequently, the less subjective method of measurement has been developed. It is called the “portable sulphide monitor” and has been well known as Halimeter®.

Rosenberg et al. (1991) compared the efficiency of the sulphide monitor with the organoleptic measurement. Although the assessment of steady-state sulphide levels by the sulphide monitor is not a direct measurement of oral malodor, it has good correlation with organoleptic method and superior reproducibility, objectivity and sensitivity. Therefore, this type of machine is widely used by several researchers. Its function is to measure the concentration of sulphur content in the air sample from the oral cavity of the subject. After gas measurement, the concentration will be compared with the standard or normal sulphur concentration range to diagnose the malodor of that person. Furne et al. (2002) investigated the accuracy of the Halimeter® compared to gas chromatography. The Halimeter® slightly overestimated the concentration of VSCs during the plateau phase (22 ± 4.0%) more than the gas chromatography. The Halimeter® could detect hydrogen sulphide (highest sensitivity), methyl mercaptan and dimethyl sulphide. Although the plateau phase measurement of the Halimeter® was 25% greater than that of gas chromatography, the gas chromatography measurement of initial total VSCs in breath samples was 2.7±0.48 times higher than the peak concentration of the Halimeter®. Even though significant differences were observed, gas chromatography and Halimeter® measurements still showed positive correlation.

Despite the beneficial features formerly mentioned, some disadvantages still occur. Sulphide monitor cannot differentiate individual gas in details and also does not detect only mouth air VSC measurement. Instead, it is also sensitive to non-malodor volatile compound (Furne et al. 2002). Mercaptan, a pungent foul-smelling sulfur compound, may not be recorded promptly as hydrogen sulphide. The analysis may be misleading especially if the individual has methyl mercaptan in substantial amounts. Moreover, the Halimeter® is very sensitive to alcohol and if one rinses the mouth with an alcohol-containing mouthwash, the reading would be quite high. Additionally, the Halimeter® shows a loss of sensitivity over time to the sulfur compounds and the machine must be frequently cleaned and recalibrated.

In 1970's, the machine called gas chromatography (GC) was developed using the flame photometric detector (FPD) to differentiate and analyze the concentration of VSCs in the air sample. This type of machine can be used to accurately detect the gas components of VSCs. This machine is considered to be the gold standard for objective measurement of oral VSCs. However, GC is not practical for chair-side clinical use because it requires a costly large-scaled system, a long measurement time and an experienced operator.

At present, a new type of gas detector is developed as the semiconductor gas sensor in the system of simplified gas chromatography. This type of gas detector is portable and can be used by chair-side. It can detect the small amount of VSCs in the ppb range and can differentiate three major VSC component which are hydrogen sulphide, methyl mercaptan and dimethyl sulphide. This simplified gas chromatography named as "Oral Chroma®" is selected to be used in this study.

Treatment of halitosis

There are many ways to treat halitosis. Mechanical cleansing can eliminate tongue coating and reduce putrefaction that may occur in oral cavity especially on dorsum of tongue. Dental caries also plays an important role because it is a reservoir for bacterial growth. Periodontal therapy seems to be important to clean out the bacterial substrate and bacterial colonization that produce VSCs. However, in moderate periodontitis patient, initial periodontal therapy and tongue scraping may have only weak impact on VSC level (Quirynen et al., 2005).

The use of chemical agents is another approach to treat halitosis. The objective is to eliminate bacteria. The form of chemical delivery commonly used is mouthrinse. The most popular chemical agent is chlorhexidine mouthrinse. Cetylpiridinium chloride, sodium fluoride, zinc chloride and zinc lactate are also used in this purpose of VSC reducion. These chemical agents are commonly used in combinations. Roldan et al. (2005) studied

efficiency of several chemical mouthrinse in combinations in 10 patients with moderate periodontitis. The results were followed up to 5 h and halitosis was measured by the Halimeter® which detect overall intraoral VSCs. The baseline values ranged from 190 to 227 ppb. At 5 h, the VSCs decreased to 155 ppb in combination of chlorhexidine and cetylpiridinium chloride and VSCs decreased to 169 ppb in combination of chlorhexidine and zinc lactate.

Quirynen et al. (2005) studied the VSC reduction by combination of periodontal therapy and chemical mouth rinse. Forty five patients with moderate periodontitis were enrolled in the experiment. They were delivered with randomly assigned mouth rinse and followed up to 6 months. The results demonstrated that combination-mouthrinse with mechanical treatment help reduce halitosis efficiently.

Essential oils are also interesting. Fine et al. (2000) examined the role of twice-daily rinsing with essential oils (Listerine®) on levels of recoverable *Streptococcus mutans* and total streptococci in supragingival interproximal plaque and in saliva. Twenty-nine subjects were randomly assigned to Listerine® as a test mouthrinse and sterile water. Subjects rinsed twice daily for 11 days and once on the twelveth day on which the microbiological quantification of recoverable *S. mutans* and total streptococci was performed. Rinsing with Listerine® demonstrated significant reductions in both *Streptococcus mutans* and total streptococci in supragingival interproximal plaque and in saliva. In addition, *in vivo* studies of essential oil-containing mouth rinse (Listerine®) inhibition effect upon plaque bacteria were also shown by Pan et al. (2000) and Charles et al. (2000).

Nutrition controls have also been widely mentioned. Even though adequate clinical experimental studies have not been performed, there were several possible reasons that seem to support relationship between malodor and change of nutrient availability. There were evidences that children with caries-free had malodor while that with high caries activity were less malodorous (Paryavi-Gholami, Minah , and Turng, 1999). High protein diet tends to play an important role in this aspect (Loesche and Kazor, 2002). While high fermentable

carbohydrate diet promotes growth of Streptococci species, the Gram-negative anaerobic bacteria especially Prevotella and Porphyromonas species survive upon high protein diet. Gram-negative anaerobic bacteria are prominent in people with food retention in interproximal area of teeth. Malodor also relates to pH condition. While *R. mucilagenous,* which are VSCs producers, are prominent in the more basic pH, streptococci which are unlikely to correlate with malodor are prominent in acidic pH (Loesche and Kazor, 2002).

Consequently, there were relationship among high protein diet, Gram-negative anaerobic bacteria, pH condition and malodor. This is not probable that bacteria degrade protein or glycoprotein-containing food during food transit through the mouth. The short period and lack of enzyme to degrade high molecular weight protein into peptide, that bacteria can use, is impossible for the bacteria to produce malodor directly from these foods. Instead, studies in animals demonstrated that the high protein diet produce high peptide in serum, saliva and gingival crevicular fluid. If the increments of peptides in saliva related to high protein diet are confirmed in human studies, then Gram-negative anaerobic bacteria can use these peptides to produce malodor (Loesche and Kazor, 2002). However, higher fermentable carbohydrate diet may activate caries formation in particular with sucrose-containing food. Consequently, adjustment of food in the aspect of high or low protein diet should be considered meticulously to inhibit the malodor formation without promoting caries activity. Maltose which is lower caries promoting than sucrose or even vegetable-rich food may be helpful.

The green tea

Tea is a water extract of leaves, blossoms, roots, bark, or other parts of tea plants. The extraction can be done by soaking, boiling and steeping (soaking in water below the boiling point). Although, they come from the same species of plant called *Camellia sinensis,* tea can be divided into many types such as white tea, green tea, Oolong (red) tea, black tea, puer tea, pouchong tea and scented (flavored) tea depending on source location, producing process, color, odor and taste. Fresh green tea beverage is tinted apple green. Other teas from *Camellia sinensis* are black, red and yellow according to the appearance of either the dried leaf or its extract. The extract can be an ordinary beverage or a medication. Black tea is the full fermented tea and has the good taste. However, it has the least beneficial ingredient among all kinds of the tea. Oolong tea is the semi-fermented tea that has some beneficial polyphenol agents. Green tea is the unfermented tea processing by steaming and avoiding oxidative reaction of polyphenol and flavonol agents. Chinese legend attributes the accidental discovery (around 2700 B.C.) of drink made from tea plant to King Shen Nong. Shen Nong most probably drank green tea, which quickly became the most popular beverage in China, Japan, Korea and the countries of Southeast Asia. Its popularity has continued and in fact, tea brewed from *Camellia sinensis* is second only to water as the world's most popular beverage.

Ingredients of green tea

The important ingredients in green tea are the polyphenol compounds that are catechin and flavonol. The catechins in green tea are epicatechin (EC), (-)-epicatechin-3-gallate (ECG), epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG) which has the highest percentage of them all (60% by weight). In addition, green tea also contains gallotannic acid, cafeine, theobromine, theophylline, esters, xanthine, β-carotine, chlorophyll, thiamine and vitamin-E.

Tea Processing

All *Camellia sinensis* teas are from the growing ends and buds (called the flushes) of the tea tree or shrub. Flushes which are undergone through a process called fermentation become black, red, or yellow teas depending on the percentage of fermentation. This process is not the same as which microbes are added to make alcohol-containing beverages, cheese, sauerkraut and other foods. Rather, an enzyme (catalyst) was added to change molecules called polyphenols which are green color into more complex polyphenols which are red and yellow colors. Both the enzyme and the polyphenols are in (and not added to) the tea leaf and leaf fermentation is started by withering (slow drying of the leaves) and then by rolling (pressing the leaves so that the sap comes to the surface). To make Black tea, the fresh tea leaves are allowed to totally ferment (100 percent). Partial fermentation of 10 to 15 percent and 20 to 30 percent yields yellow and red (sometimes known as Oolong) teas, respectively. Green tea is produced by steaming or roasting the leaves to inactivate the enzymes soon after harvested to prevent fermentation and therefore it contains catechins which seem to be the most important and interesting ingredient. A cup (200 ml) of green tea (Gun Powder, Hangzhou, China) contains 142 mg EGCG, 65 mg EGC, 28 mg ECG, 17 mg EC and 76 mg caffeine.

Epigallocatechin-3-gallate (EGCG)

EGCG is the beneficial form of catechin with the molecular formula of $C_{22}H_{18}O_{11}$ (Figure 4) and seems to be the most effective catechin being studied nowadays. EGCG has some beneficial properties found in many studies as can be demonstrated.

Hofmann and Sonenshein (2003) studied the effect of EGCG on smooth muscle cell from aorta and found the antiproliferative effect at concentration of 40-50 μg/ml and apoptosis inducing effect at concentration of 80 μg/ml and consequently these properties bring about the ability to prevent the atherosclerosis.

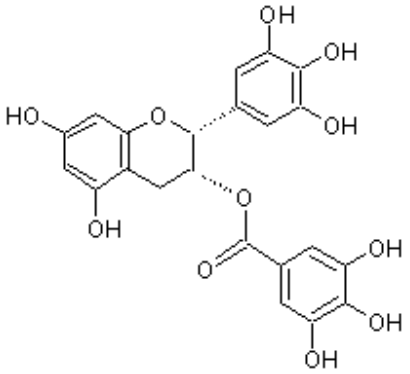


Figure 4: Molecular structure of EGCG

The EGCG was demonstrated to inhibit bacterial cellular growth and inhibit epithelial cell adhesion by *Porphyromonas gingivalis.* (Sakanaka et al., 1996). The EGCG was also found to inhibit protein tyrosine phosphatase of *Prevotella intermedia* by the galloyl moiety of the EGCG molecular structure (Okamoto et al., 2003). The EGCG was also proved to be useful for its inhibitory effect on periodontal disease progression (Makimura et al., 1993). EGCG has bactericidal property by formation of reactive oxygen species (ROS). The EGCG reacts with oxygen in aqueous solution to produce hydrogen peroxide which is toxic to bacteria (Arakawa et al., 2004). Hydrogen peroxide derived from this catechin rose with increasing pH. Bactericidal property depends upon hydrogen peroxide level generated by EGCG.

EGCG contains gallo radical which has an antibacterial property and among the polyphenol agents in Oolong tea extract, EGCG is the most effective antibacterial agent (Sakanaka et al., 1989). EGCG can destroy bacterial lipid bilayer, bind to peptidoglycan and inhibit enzyme activities (Okamoto et al., 2004). Furthermore, upon the study of Arg-gingipain (Rgp) -deficient mutant and Lys-gingipain (Kgp) -deficient mutant, EGCG can inhibit the cysteine proteinase activity, the virulence factor of the *Porphyromonas gingivalis,* in the same manner as chlorhexidine gluconate. In addition, EGCG also has inhibitory effect on glucosyltransferase activity (Hattori et al.1990) and its effect is higher than other polyphenolic compounds (Otake et al., 1991).

EGCG also has inhibitory effect on other bacteria. It inhibits growth of *Helicobacter pylori* at MIC of 50-100 μg/ml (Yee and Koo, 2000) and can dose-dependently inhibit the growth of methicillin-resistant *Staphylococcus aureus* (Zhao et al., 2001). Moreover, there is the effect of green tea catechin on *Prevotella intermedia*'s protein tyrosine phosphatase, which is the potent enzyme interfering with the macrophage function. EGCG can inhibit protein tyrosine phosphatase activity in *Prevotella nigrescense* at 5 μM, *Prevotella pallens* at 5 μM and *Prevotella intermedia* at 0.5 μM. However, EGCG cannot inhibit this enzyme activity in *Porphyromonas gingivalis* which has different kind of protein tyrosine phosphatase (Okamoto et al. 2003).

Additionally, Oolong tea which contains EGCG has been reported to inhibit enzyme-synthesizing water-insoluble glucan glucosyltransferase-1 (GTF-1) of *Streptococcus sobrinus* 6715 and cell-associated GTF of *Streptococcus mutans* MT8148R (Nakahara et al. 1993). Also EGCG in Oolong tea can inhibit plaque adherence of *Streptococcus mutans* to glass surface in culture medium. Green tea extract is demonstrated to inhibit growth of oral bacteria especially *Porphyromonas gingivalis* and *Fusobacterium nucleatum* at 37 °C, 10%CO_2, 10%H_2, and 80%N_2 (Saeki et al. 1993).

. Furthermore, EGCG also has inhibitory effect on host tissue destruction caused by matrix metalloproteinases (MMPs). MMP is the family of proteolytic enzyme that degrades collagen, gelatin and elastin. MMP-1 and MMP-8 are collagenase. While MMP-8 results from inflammatory neutrophils, MMP-1 comes from resident cells such as fibroblasts and epithelial cells (Newman et al., 2000). MMP-9 also plays an important role in tissue destruction. Yun et al. (2004) showed that treatment with the sonicated *Porphyromonas gingivalis* extracts stimulated the expression of MMP-9 mRNA and this effect was significantly reduced by EGCG. On contrary, the transcription levels of MMP-2 and MMP-13 were not affected by either the sonicated *Porphyromonas gingivalis* extracts or EGCG. In addition, EGCG significantly inhibited osteoclast formation in the co-culture system at a concentration of 20 μM. Therefore, it can be concluded that EGCG may prevent the

alveolar bone resorption that occurs in periodontal diseases by inhibiting the expression of MMP-9 in osteoblasts and the formation of osteoclasts.

Moreover, the steric structure of 3-galloyl radical is important for the inhibition of collagenase activity. The collagenase activity in the gingival crevicular fluid from highly progressive adult periodontitis is completely inhibited by the addition of tea catechins. These results demonstrated that tea catechins containing galloyl radical possess the ability to inhibit both eukaryotic and prokaryotic cell derived collagenase (Makimura et al., 1993). Green tea catechin is demonstrated to have a bactericidal effect against black-pigmented and gram-negative rod anaerobic bacteria. When used in combination with mechanical treatment i.e. scaling and root planning, green tea catechins, as the local delivery approach in the periodontal pocket, can improve periodontal status. Hirasawa et al. (2002) performed a clinical pilot study which used hydroxypropylcellulose strip containing green tea catechin as bactericidal agent. The strip was introduced into periodontal pocket, and periodontal status was recorded. The results demonstrated the improvement of periodontal status and the reduction of the black-pigmented gram negative anaerobic bacterial proportion after using the green tea catechin strip.

Toxicity of EGCG

Green tea extract and its principal active ingredient, epigallocatechin gallate (EGCG), are becoming very popular today due to their healthful properties. Despite the increasing demand for these products, safety still has to be studied. The toxicity of purified green tea extracts containing high concentrations of EGCG have been evaluated in some studies in order to define the safety of EGCG extract. Topical EGCG is shown to cause minor dermal irritation in rats and guinea pigs, but not rabbits, and was a moderate dermal sensitizing agent in the guinea pig. An oral dose delivering 2000 mg EGCG /kg was lethal to rats (Isbrucker et al. 2006) whereas, a dose of 200 mg EGCG/kg had no toxicity. The dietary administration of EGCG to rats for 13 weeks was not toxic at doses up to 500 mg/kg/day. Moreover, no adverse effects were noted when 500 mg EGCG /kg/day was administered to pre-fed dogs in divided doses. However, this dose caused morbidity when administered to

fasted dogs as a single bolus dose. In the experimental study on the effect of EGCG on normal rat fetal development, the dose of EGCG in the safety level (no-observed adverse effect level) was equivalent to 200 mg/kg/day EGCG (Isbrucker et al. 2006).

CHAPTER III

MATERIALS AND METHODS

Populations

The populations in this study were periodontal patients recruited from the Graduate Clinic of Department of Periodontology, Faculty of Dentistry, Chulalongkorn University with the following criteria:

- were diagnosed as chronic periodontitis
- no history of systemic conditions nor taking antibiotics or other medications which may cause confounding effects on oral malodor measurement
- no history of periodontal treatment on the past 6 months

The subjects who were eligible for this study needed to pass the screening malodor measurement at not less than 125 ppb of total VSC gas level. There were 30 subjects who met these criteria participated in this study.

Materials and Methods

The effect of EGCG-3-gallate from green tea on malodor was evaluated with the double-blinded randomized controlled crossover clinical experiment. This study was approved by the Ethics Committee of Faculty of Dentistry, Chulalongkorn University. Each subject received the experiment information and then signed the written consent form before starting the study.

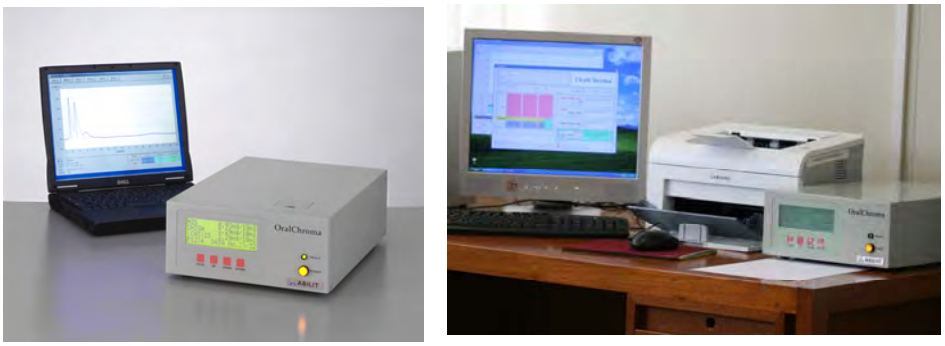


Figure 5:The Oral Chroma®

Materials

1. Oral Chroma® equipment (Abilit, Japan) (Figure 5)
2. one-ml disposable plastic syringe (Nipro®)
3. injection needles gauge No.23
4. Test reagent was 50 mg/100 ml EGCG (95% purity, Sigma-Aldrich (USA)) in double distilled water.
5. The control was double distilled water.

Methods

1. The agents were randomly delivered to each subject. Both the control and test agents were colorless and were covered with foil to avoid bias.
2. The researcher A was assigned only to measure individual oral VSC at each time point and was kept out of mouth rinsing process without knowing the type of mouth rinse for each subject.
3. The researcher B was assigned to deliver the mouth rinsing agent directly to the subject and control the rinsing process.
4. The experiment was performed randomly for all 30 subjects between the test and control agent and then crossover with one week washout period.
5. All subjects were informed that the experiment was planned to start from 7.00 AM to 11.00 AM. They were asked to strictly follow this protocol:
    - the denture wearers had to refrain from wearing their dentures since the night before and on the experiment day.
    - in the morning of the experiment day, they had to refrain from all types of oral hygiene procedure and abstain from any food and beverages and from using any cosmetic or aromatic agents which may have confounding effect on the gas measurement.
    - In one day, up to three subjects were measured for gas level and the Oral Chroma® was set up 30 min everyday before measurements started.
6. The experiment procedure was begun with the odor sampling. Each subject was asked to close his/her mouth for 3 min. Then the mouth odor was collected in a

plastic syringe and the baseline VSC concentration was measured with Oral Chroma® (Figure 6).

7. The subject was delivered 15 ml rinsing agent to rinse his/her mouth for 2 min and then spit it out without water rinse. Fifthteen min after rinsing, the oral VSC concentration was measured again. The measurements were repeated at the following time points: 30, 60 and 120 min after rinsing.

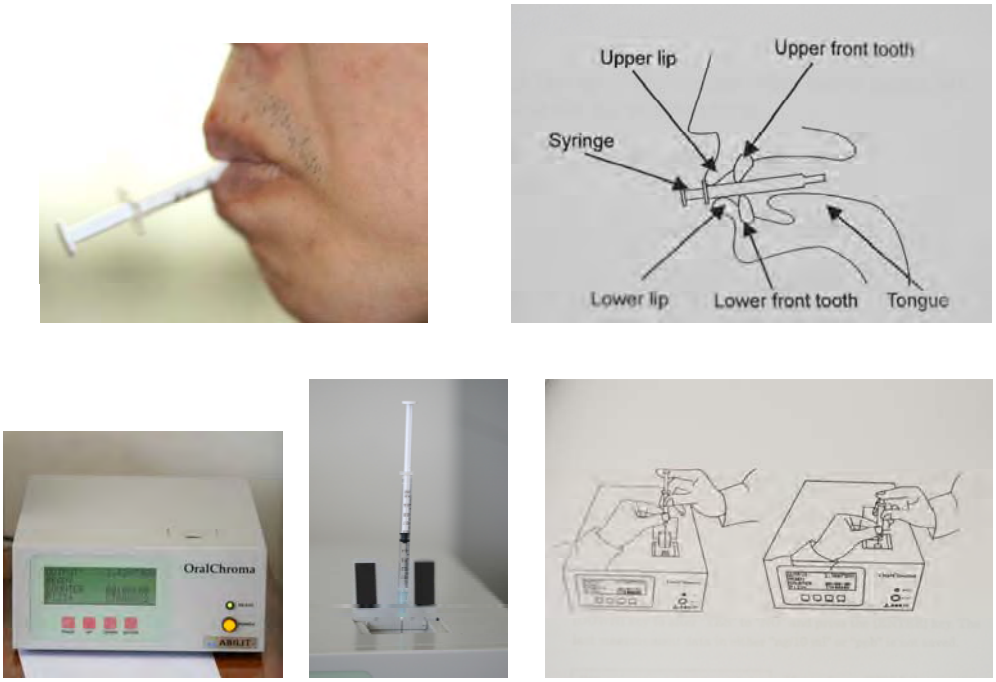


Figure 6: Mouth air collection

Data analysis

The concentration of three volatile sulphur gases obtained at each time point after the rinse was calculated as gas change from baseline level.

The statistical decision was determined at α =0.05 (95% confidence interval). VSC level of each gas component was presented by mean or median and the comparisons were analyzed by Student's t-test or Wilcoxon signed ranks test depending on the statistical normal distribution of the data. However, almost all gas change data showed statistical

normal distribution except for the data of methyl mercaptan gas change at 30 min. Then Wilcoxon signed ranks test was used.

CHAPTER IV

RESULTS

Thirty subjects who were diagnosed as periodontitis and had VSCs concentration not less than 125 ppb were enrolled in this study. They were 4 male and 26 female with age ranged from 16-59 years (mean=38.7 ± 11.6) (Table 1). They were randomly assigned to mouthrinse delivery sequence. No subject dropped out. No adverse effects in the oral cavity during the experiment had been reported.

The baseline of total VSCs gas level ranged from 128 to 4,484 ppb. Mean baseline total VSCs gas level of the control and test agents were 697±927 and 784±876 ppb, respectively. When the total VSCs gas level within the test group at each time point was concerned, the test mouthrinse showed statistically significant reduction from baseline at 15, 30, 60 and 120 min. The gas level of hydrogen sulphide, methyl mercaptan and dimethyl sulphide decreased significantly from baseline until 15, 30, 60 and 120 min, respectively (Table 2). However, to evaluate the efficacy of the test mouthrinse, its ability to reduce the total VSCs gas level was compared with the control agent. Due to the great variation in the gas level among individuals, the changes of gas level according to the mouthrinse was presented in the form of percentage change from baseline value.

The total gas level, in almost all of the subjects, in the control and test mouthrinse groups decreased rapidly from the baseline after 15 min rinse and then relatively slowly increased with time. However, there were no significant differences for the mean total gas level between the control and the test groups at almost all of the time points ($P > 0.05$). However, EGCG presented its potential for total VSCs gas reduction over the control agent at 30 min ($P=0.039$)(Table 3 and Figure 7).

When each component of the VSCs was concerned, percentage of gas change from baseline between the test and control group was compared. For hydrogen sulphide gas level, its baseline ranged from 14 to 1,547 ppb. Mean baseline hydrogen sulphide gas level of the control and test agents were 352±423 and 440±490 ppb, respectively. Medians of baseline hydrogen sulphide gas level of the control and test agents were 179 and 235 ppb, respectively. No statistically significant difference of baseline hydrogen sulphide gas

level between the control and the test agent was found (*P*=0.141). Because of the great variation in the gas level among individuals, the change of gas level according to the mouthrinse was presented in the form of percentage change from baseline value.

The hydrogen sulphide gas level, in almost all of the subjects, in the control and test mouthrinse groups decreased rapidly from the baseline after 15 min rinse and then relatively slowly increased with time. However, there were no significant differences for the mean hydrogen sulphide level between the control and the test groups at any time points (*P*> 0.05) (Table 4 and Figure 8).

The baseline of methyl mercaptan ranged from 14 to 2,624 ppb. Mean baseline methyl mercaptan gas level of the control agent was 281±511 ppb while that of the test agent was 267±370 ppb. Although median value of methyl mercaptan baseline level of the control agent was 136 ppb, which was quite different from that of the test agent (97 ppb), they were not significant different (*P*=0.869).

Similar to hydrogen sulphide, immediately after mouthrinsing, the methyl mercaptan level rapidly dropped at median of 31.53% and 74.17 % in the control agent and the test agent respectively after 15 min and then slowly increased with time. At 15 min after mouthrinsing, the difference of gas level reduction was not statistically significant (*P*=0.063).

However, at 30 min after mouth rinsing, the medians of gas level reduction of the control and the test group were 3.42% and 67.89% respectively, and it was significantly different (*P*=0.000). Furthermore, at 60 min after mouth rinsing, the test group still showed superior ability in gas reduction (43.36%), while the control group became lose most of gas reduction ability (*P*=0.029). The percentage of gas change level in the control group increased 10.79% from baseline.

These similar results continued to the 120 min time point. The mean gas level of methyl mercaptan of the control group increased 42.28±103.17%, while that of the test group was still less than baseline 37.00±63.77%. The statistically significant difference between the control and test groups for methyl mercaptan gas level at 120 min was found (*P*=0.002) as at 30 and 60 min. The details were shown in Table 5 and Figure 9.

For the dimethyl sulphide gas level, its baseline ranged from 0 to 360 ppb depending on individual physiological condition. Because there was minimal variation in the gas level among individuals, the change of the gas level according to the mouthrinse was presented by simple subtraction from the baseline value. Mean baseline dimethyl sulphide gas level of the control and test agents were 63±68 and 77±77 ppb, respectively. Statistical analysis demonstrated no statistically significant difference of baseline dimethyl sulphide gas level between the control and test agents (*P*=0.513).

The dimethyl sulphide gas level in almost all of the subjects both in the control and test mouthrinse groups decreased rapidly from baseline after 15 min rinse and then relatively slowly increased with time. However, there were no statistically significant differences for the mean dimethyl sulphide level between the control and test groups at any time points (*P*> 0.05). The details were presented in Table 6 and Figure 10.

When the effect of the test mouthrinse on VSCs gas change level was concerned, the mean or medians of VSCs gas level were analysed as shown in Table 6. The hydrogen sulphide gas level reduced significantly at 15, 30 and 60 min (*P*=0.000, *P*=0.001 and *P*=0.005, respectively), however, no significant reduction was found at 120 min (*P*=0.465).

For methyl mercaptan gas level, the test mouthrinse demonstrated statistically significant reduction at all time points (*P*=0.000, *P*=0.001, *P*=0.001 and *P*=0.000 at 15, 30, 60 and 120 min, respectively).

For dimethyl sulphide gas level, there were statistically significant reduction at 15 min (*P*=0.045) and again at 60 min (*P*=0.031). However, there were no statistically significant gas reductions at 30 min (*P*=0.241) and 120 min (*P*=0.690).

Table 1 Demographic information of the subjects

Age (y)	
Mean (SD)	38.7 (11.6)
Range	16-59
Gender	
Male	4 (13.3%)
Female	26 (86.7 %)

Table 2 Comparison of VSC level between baseline and post-mouthrinse periods in test group ("+" = gas increment, "-"= gas reduction)

VSCs	0 min	15 min	30 min	60 min	120 min
Hydrogen sulphide (mean)	440	256	262	281	357
*P-value**		0.000	0.001	0.005	0.465
Methyl mercaptan (mean)	267	149	171	183	144
*P-value**		0.000	0.001	0.001	0.000
Dimethyl sulphide (mean)	77	64	73	60	67
*P-value**		0.045	0.241	0.031	0.690
Total VSCs (median)	452	205	188	301	326
*P-value***		0.000	0.001	0.000	0.021

* Student's t-test

** Wilcoxon signed ranks test

Table 3 The mean of total VSCs gas level change

("+" = gas increment, "-"= gas reduction)

	Gas change of total VSCs from baseline							
	15 min		30 min		60 min		120 min	
	control	test	control	test	control	test	control	test
Mean	-30.05	-35.31	-6.69	-36.94	1.33	-26.08	21.85	-5.90
Std. Deviation	47.28	59.09	50.69	47.29	56.78	56.61	70.92	72.33
P-value	0.73		0.039		0.094		0.162	

Table 4 The percentage of mean and median of hydrogen sulphide gas level change

("+" =gas increment, "-" = gas reduction)

	Gas change from baseline (%)							
	15 min		30 min		60 min		120 min	
	control	test	control	test	control	test	control	test
Mean %	-31.45	-32.21	-18.15	-28.92	6.16	-8.72	18.95	18.83
Std. Deviation	55.63	45.71	63.17	63.21	88.93	86.78	113.35	95.09
Median %	-51.82	-19.09	-35.33	-38.87	-11.43	-42.11	0	-5.92
P-value	0.956		0.486		0.507		0.996	

Table 5 The mean and median of percentage of methyl mercaptan gas level change

("+" = gas increment, "-"= gas reduction)

	Gas change from baseline (%)							
	15 min		30 min		60 min		120 min	
	control	test	control	test	control	test	control	test
Mean %	-24.28	-54.70	17.95	-53.94	18.95	-29.27	42.28	-37.00
Standard deviation	56.90	47.79	123.92	43.40	75.63	68.46	103.17	63.77
Median %	-31.53	-74.16	-3.42	-67.89	10.79	-43.36	17.89	-50.37
P-value	0.063		0.007		0.029		0.002	

Table 6 The mean and median of dimethyl sulphide gas level change

("+" = gas increment, "-"= gas reduction)

	Gas change from baseline							
	15 min		30 min		60 min		120 min	
	control	test	control	test	control	test	control	test
Mean	-21.23	-12.40	.40	-3.96	14.33	-17.50	18.63	-10.06
Std. Deviation	45.66	88.80	52.15	66.30	71.53	53.740	65.79	79.88
Median	-7.50	-24.00	4.00	-12.50	16.00	-11.50	18.00	0
P-value	0.659		0.817		0.074		0.213	

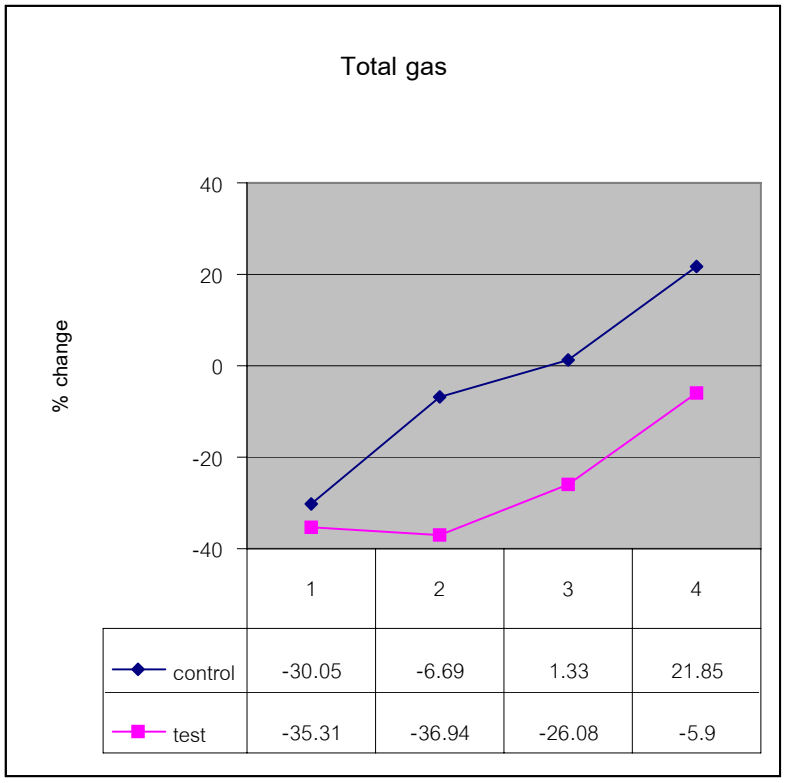


Figure 7: Effect of the test (EGCG) mouthrinse and the control on total VSC gas level presented as % gas change from baseline at 15 (1), 30 (2), 60 (3), 120 (4) min.

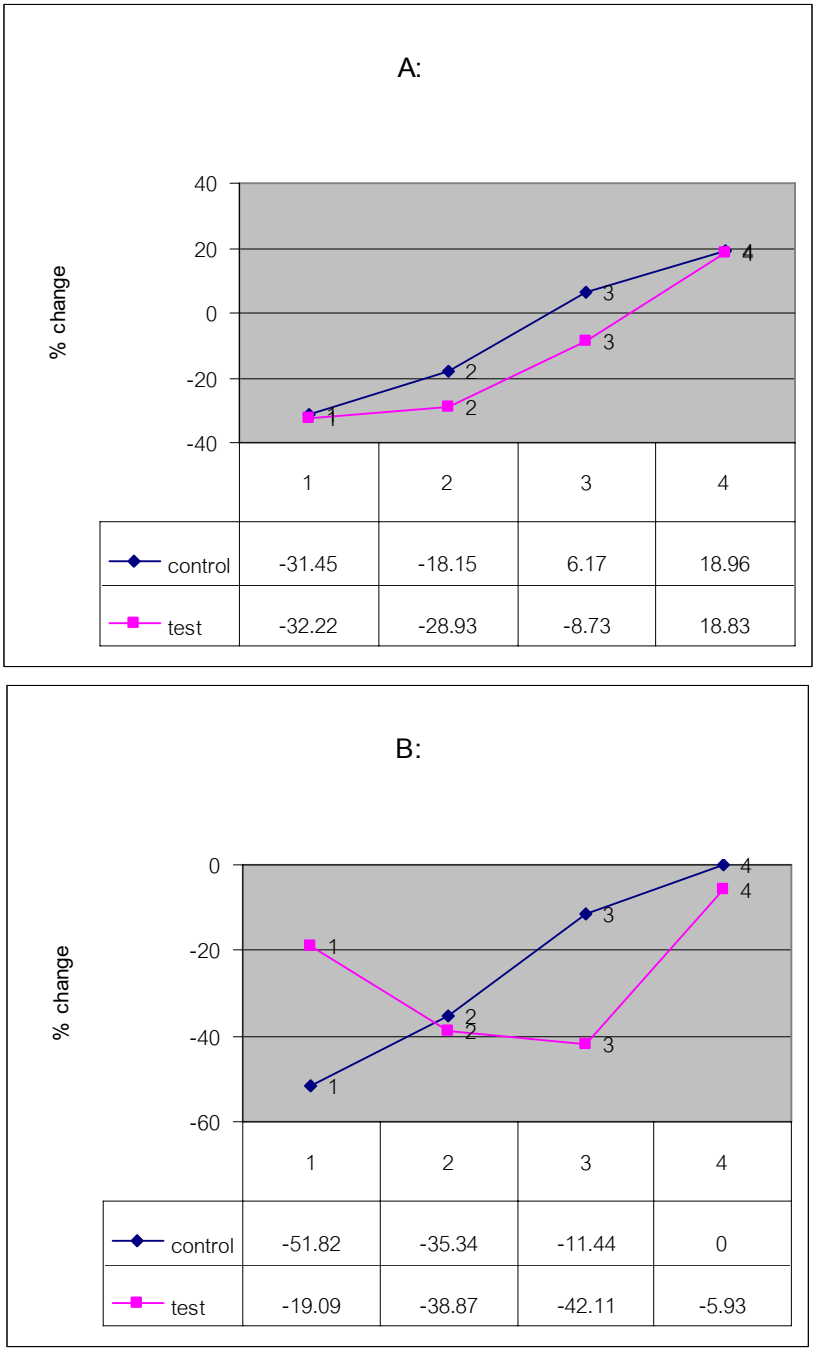
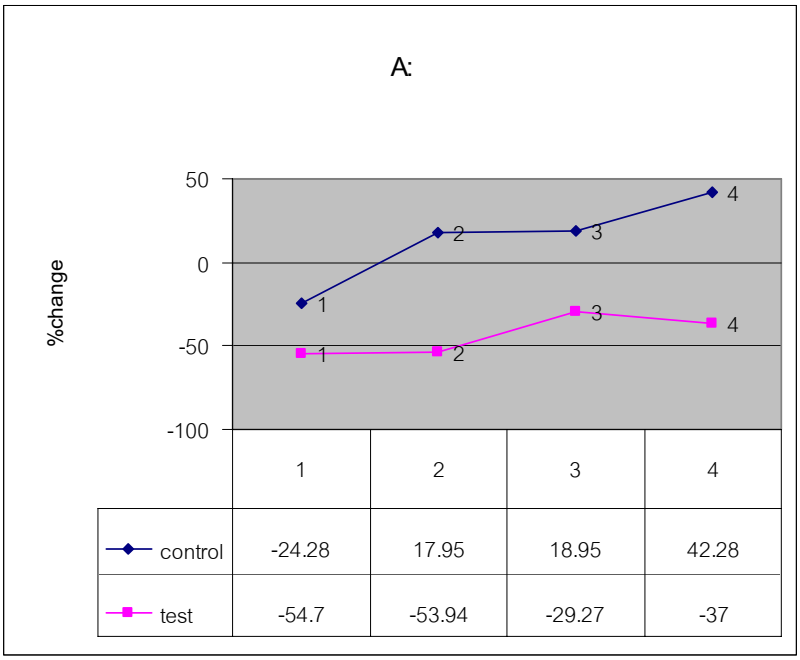


Figure 8: Effect of the test (EGCG) mouthrinse and the control on hydrogen sulphide gas level presented as % gas change from baseline at 15 (1), 30 (2), 60 (3), 120 (4) min.

A: mean value

B: median value



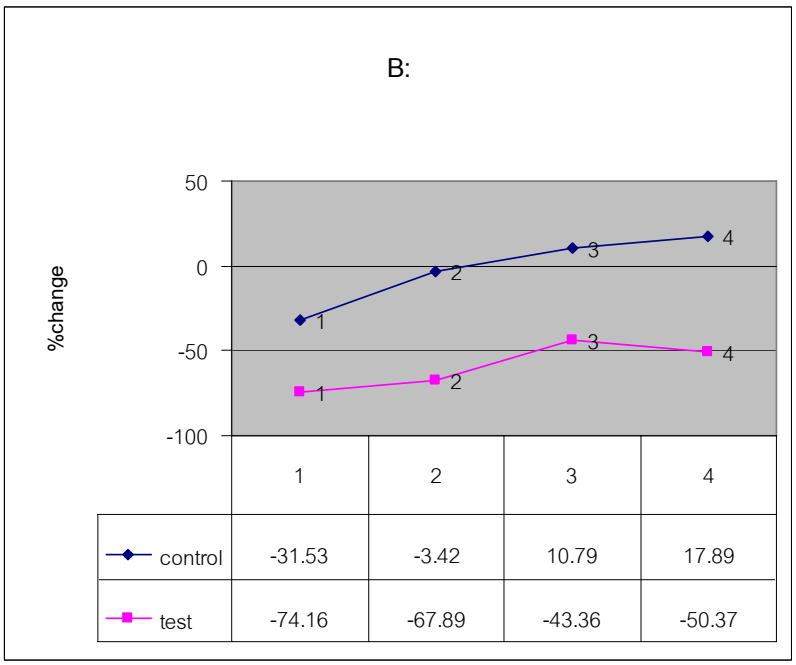


Figure 9: Effect of the test (EGCG) mouthrinse and the control on methyl mercaptan gas level presented as % gas change from baseline at 15 (1), 30 (2), 60 (3), 120 (4) min.

A: mean value

B: median value

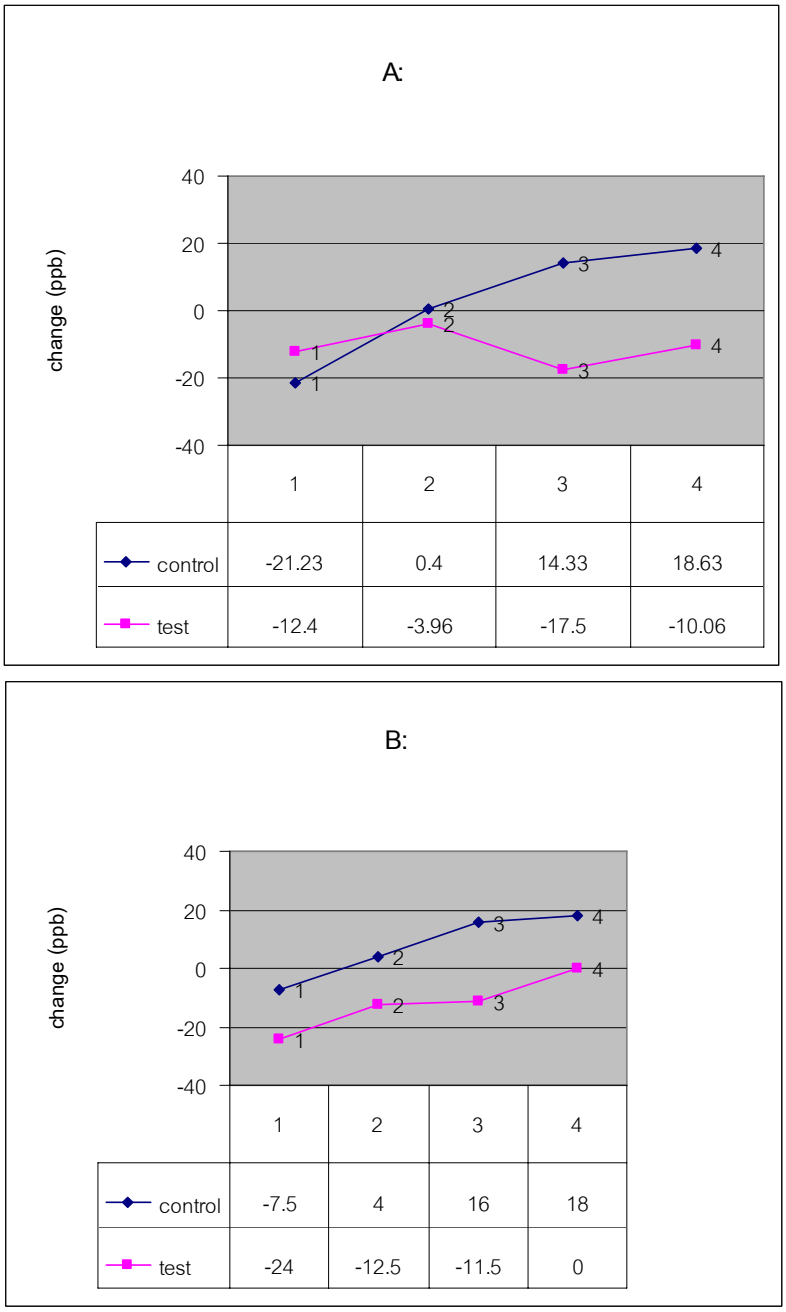


Figure 10: Effect of the test (EGCG) mouthrinse and the control on dimethyl sulphide gas level presented as % gas change from baseline at 15 (1), 30 (2), 60 (3), 120 (4) min.

A: mean value

B: median value

CHAPTER V

DISCUSSION

Oral malodor is the most common self reported problem for many people. It results from several types of gas produced in the oral cavity eg. volatile amines. However, the major malodor-producing gases are VSCs (Tangerman, 2002). Many researchers have attempted to find out the appropriate treatment of this problem. To seek for the effective chemical agent, one needs to well understand the component of these gases and knows the mechanism of gas production. Moreover, individual gas has to be considered separately because they may have significant effects on the efficacy of malodor treatment.

EGCG has been reported in a large number of studies to have antimicrobial property. EGCG has bactericidal property against a variety of microorganism such as *Helicobacter pylori* (Mabe et al., 1999) and inhibits extracellular release of toxin from enterohemorrhagic *Escherichia coli* O157-H7 (Sugita-Konishi et al., 1999). EGCG is also found to have inhibitory effect upon oral bacteria such as *Prevotella intermedia* (Okamoto et al., 2003)*, Porphyromonas gingivalis* (Okamoto et al., 2004) *and Streptococcus mutans* (Ooshima et al., 1994). These bacteria result in putrefaction in the oral cavity and produce gases including VSCs.

Therefore, it is reasonable to investigate the clinical application of EGCG, a major polyphenol compound in green tea. This clinical experiment aimed to study the efficacy of EGCG on the reduction of the three major VSCs found in oral cavity. The EGCG was used in the form of the mouthrinse which required high concentration to have more effective antimicrobial property. The EGCG concentration used in this study was 50 mg/100 ml which was 10 times of the minimal inhibitory concentration to inhibit *Porphyromonas gingivalis.*

The novelty of this research was the investigation of individual component of VSCs by the Oral Chroma®, the simplified gas chromatography machine, which can measure VSC concentration and can differentiate the VSC into three major components. These are hydrogen sulphide, methyl mercaptan and dimethyl sulphide. Comparing to the Halimeter®

which was used in several studies, the Oral Chroma® may have more benefits in searching for the effective treatment modalities for oral malodor.

However, this study did not use organoleptic measurement which was widely used for malodor evaluation. It was because this study primarily focused on the three major VSC component changes in periodontitis patients related to the intervention of mouthrinse agent. The organoleptic method, however, measured the total gas level which is not suitable for this study. So it was not necessary to involve the organoleptic measurement which may be at risk for respiratory disease transmission from patient to researcher.

From the result of this study, EGCG presented its potential to reduce total VSCs gas level until 120 min when compared to baseline, however, its efficacy over the control agent was found only at 30 min. Because major oral VSCs are composed three major gases; hydrogen sulphide, methyl mercaptan and dimethyl sulphide, it is interesting to evaluate the efficacy of EGCG on each VSCs component to find out the mechanism to reduce malodor.

Due to the variations of oral conditions and bacterial environment among individuals, the gas reduction of hydrogen sulphide and methyl mercaptan showed high standard deviations. Different diet of each person may also be one factor related to these variations, because it directly associated with the substrate being used by bacteria (Loesche and Kazor, 2002). However, the inclusion criteria of this study had focused on chronic periodontitis patients who had at least 125 ppb total VSC gas level which is the threshold of VSC that can be recognized as malodor. Therefore, the hydrogen sulphide and methyl mercaptan gas level were computed in the form of percentage gas change. However, for dimethyl sulphide, the variation was not as much, and some baseline gas level was zero, so the change of this gas could not be calculated in percentage change. The mean value of this gas was then used to demonstrate the efficacy of the mouthrinse.

For hydrogen sulphide, the EGCG mouthrinse decreased the gas level rapidly at 15 min after mouthrinsing. At 30 min, the gas level tended to increase relatively slowly until 60 min in which time hydrogen sulphide gas levels were still statistically significantly lower than baseline value. At 120 min, however, the gas level increased to statistically similar to

baseline value. This demonstrated that the gas reduction period of EGCG was less than 2 h period.

Despite the gas reduction mentioned above, the control agent also reduced the hydrogen sulphide gas level in the pattern that was similar to the test mouthrinse. The difference between the control and test groups was not statistically significant. Consequently, this gas reduction might be from the effect of physical change from mouthrinsing activity rather than the chemical reaction to the bacteria.

Even though the EGCG is an antimicrobial agent, this ability as mouthrinse could not be demonstrated upon VSC reduction at the 50 mg/100 ml concentration. This may be due to the fact that hydrogen sulphide can be produced by several bacteria. The potent ones are *Treponema denticola* and the black-pigmented species ie. *Bacteroides intermedius, Bacteroides loescheii, Porphyromonas endodontalis* and *Porphyromonas* gingivalis (van Winkelhoff et al., 1986). Because various antimicrobial agents affect different kinds of bacteria, it was possible that 50 mg/100 ml EGCG may not be effective against all hydrogen sulphide-producing bacteria. Therefore, EGCG may not inhibit all hydrogen sulphide formation effectively. The higher EGCG concentration or other forms of EGCG should be considered to more effectively reduce hydrogen sulphide level in oral cavity.

For methyl mercaptan, similar to the hydrogen sulphide, the EGCG decreased methyl mercaptan gas level efficiently after mouthrinsing. The result was statistically significant at 15 min after mouthrinsing and the ability to inhibit this gas level continued until the period of 120 min after mouthrinsing. The statistical significances pointed out that the ability of the EGCG to inhibit methyl mercaptan gas level tended to prolong beyond the 2 h period and new experimental studies should be considered to determine the maximum extent of this agent upon this gas level reduction. Moreover, when comparing the ability in gas reduction between the control and test groups, there were statistically significant differences at almost all of the tested time points. Even though gas reduction ability of EGCG was not statistically significantly different from the control agent at 15 min after mouthrinsing, at 30 min, 60 min and 120 min, the control agent showed less gas reduction

ability than the EGCG. This suggested that the EGCG has the superior ability in gas reduction upon methyl mercaptan compared to the control agent.

The results above were supported by the fact that bacteria play an important role in producing VSC in oral cavity (Tonzetich, 1977). The VSCs result from proteolytic degradation by predominantly anaerobic Gram-negative oral microorganisms of various sulphur-containing substrates in food debris, saliva, blood and epithelial cells (Loesche and Kazor, 2002). To inhibit malodor, one has to eliminate bacteria or prevent the mechanism committed by bacteria. The EGCG is an antimicrobial agent that can fulfill this mission especially by the inhibitory effect on anaerobic Gram-negative bacteria (Okamoto et al., 2003; Sakanaka et al., 1996).

For the dimethyl sulphide gas level, the pattern of gas reduction was similar to the hydrogen sulphide. The gas levels were statistically significantly reduced from baseline gas level at 15 min and 60 min while they were not statistically significantly different from the baseline gas level at 30 min and 120 min. When considering the effect on gas reduction every time point, both the test and the control groups were not significantly different.

As mentioned above, the EGCG tended to have little effect upon dimethyl sulphide gas reduction and no effects are found beyond 1 h after mouthrinsing. This suggested that the antimicrobial property of the EGCG have only little effect on dimethyl sulphide formation by bacteria. Therefore, the reasons above seemed to explain the ineffective ability of the EGCG to reduce the dimethyl sulphide gas level in oral cavity.

After analyzing the results gained from this experimental study, it may be possible to suggest that the EGCG have an interesting property as a form of mouthrinse upon the reduction of methyl mercaptan gas level compared to distilled water alone. This chemical agent should be studied more about its appropriate concentration and its maximum extent of time to have significant effect on VSC reduction. Furthermore, it might be possible for the EGCG to inhibit hydrogen sulphide when used in the higher concentration. However, there may be some important matter, particularly the adversed effect, to be considered when the chemical agent is used with high concentration. Chow et al. (2001) studied the pharmacokinetic property of tea polyphenols following single-dose administration of the

EGCG in human. They delivered up to 800 mg EGCG to the healthy human subjects without any serious adverse effect reported. Therefore, the concentration of 50 mg/100 ml is relatively small dose. The higher concentration is still possible to be used.

While considering other chemical agent used to inhibit halitosis, chlorhexidine, as the gold standard chemical agent to inhibit periodontal pathogens, has been widely documented. However, long term use of chlorhexidine mouthrinse may bring about some side effects (Olsen, 1975). Roldan et al. (2004) demonstrated that combination mouthrinse of 0.12% chlorhexidine and 0.05% cetylpiridinium chloride has the ability in VSC reduction at 5 h. As combinations, these agents decreased side effect produced by chlorhexidine mouthrinse alone. Quirynen et al. (2005) also demonstrated efficient VSC reduction after combination of periodontal treatment, tongue scraping and chlorhexidine mouthrinsing.

True halitosis results from bacteria that can be found in posterior dorsum of tongue and deep periodontal pockets. In this clinical experimental study, subjects were periodontitis patients who have rich source of bacteria especially periodontal pathogens which are known to be the producers of methyl mercaptan. *Porphyromonas gingivalis* especially strain W83 is the active producer of this volatile gas which in turn may increase virulence of this bacteria upon host tissue destruction (Neiders et al.,1989). Other periodontal pathogens such as *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* are also methyl mercaptan producer. Consequently, methyl mercaptan is the important volatile gas to be considered in this study.

Because the EGCG has antibacterial property especially against periodontal pathogens that are mentioned above, it might be possible that EGCG can play an important role in anti-halitosis particularly for methyl mercaptan which is the gas related to periodontitis. This experimental study results demonstrated and confirmed that there were statistically differences between methyl mercaptan level in the control and that in the EGCG group. However, this experimental study was limited by small sample size and only one EGCG concentration was tested. It will be useful to continue the investigation with various

concentrations of the EGCG and larger sample size to find out the appropriate approach to use this catechin as an anti-halitosis agent for periodontitis patients in the future.

CHAPTER VI

CONCLUSION

In this study, it can be concluded that EGCG had beneficial effect in reducing methyl mercaptan which is one of the major components of VSCs in periodontitis patients. This effect was superior to distilled water. It may be due to the antimicrobial activity of EGCG upon periodontal pathogens which are the prominent methyl mercaptan producers in oral cavity. EGCG as the form of mouthrinse may have some potency in reduction of oral malodor in periodontitis patients.

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Appendices

Appendix: A

ข้อมูลสำหรับผู้ร่วมวิจัย

เรียน ผู้ร่วมวิจัยทุกท่าน

กลิ่นปากเป็นกลิ่นที่ไม่พึงประสงค์ที่เกิดขึ้นภายในช่องปาก ทำให้ผู้มีกลิ่นปากเกิดความกังวลใจและขาดความมั่นใจในการเข้าสังคม สาเหตุหนึ่งที่ทำให้เกิดกลิ่นปากเกิดจากเชื้อแบคทีเรียในช่องปากผลิตแก๊สที่เป็นสารประกอบของซัลเฟอร์ (volatile sulfur compounds) ซึ่งมีกลิ่นเหม็น น้ำยาบ้วนปากที่มีฤทธิ์ยับยั้งเชื้อแบคทีเรียอาจสามารถกำจัดกลิ่นไม่พึงประสงค์เหล่านั้นได้

ท่านเป็นผู้ได้รับเชิญจากผู้ทำการวิจัยให้เข้าร่วมในโครงการวิจัยเรื่อง ผลของของอีพิแกลโลคาเทชิน-3-แกลเลทจากชาเขียวต่อกลิ่นปาก โดยมีวัตถุประสงค์เพื่อศึกษาผลของของอีพิแกลโลคาเทชิน-3-แกลเลทจากชาเขียวต่อปริมาณแก๊สซัลเฟอร์ที่เกิดขึ้นในช่องปาก ในการศึกษานี้ต้องการผู้ร่วมวิจัย 30 คน

ในการร่วมโครงการนี้ ท่านจะต้องงดการแปรงฟันและงดการใช้ผลิตภัณฑ์ในการทำความสะอาดฟันและปากเฉพาะในวันที่ทำการทดลอง ท่านจะต้องงดอาหารและเครื่องดื่มทุกชนิด ผู้วิจัยจะทำการเก็บกลิ่นจากช่องปากของท่านโดยใช้หลอดพลาสติกขนาดเล็กสอดเข้าไปในช่องปาก และดูดอากาศในช่องปากออกมาเพื่อทำการวิเคราะห์ ท่านจะได้รับน้ำยาเพื่อบ้วนปากเป็นเวลา 2 นาที เมื่อครบเวลาให้บ้วนน้ำยาทิ้ง ผู้วิจัยจะดูดอากาศในช่องปากของท่านออกมาหลังจากท่านบ้วนแล้วเป็นเวลา 15 นาที 30 นาที 1 ชั่วโมง และ 2 ชั่วโมง ตามลำดับ ท่านจะได้พักเป็นเวลาอย่างน้อย 1 สัปดาห์ จากนั้นจึงเริ่มการทดลองใหม่ด้วยวิธีตามที่กล่าวแล้วข้างต้น ท่านจะได้รับผลตอบแทนเป็นเงินจำนวน 800 บาท เมื่อได้ร่วมทำการวิจัยจนเสร็จสิ้นการทดลอง ผลของการวิจัยในโครงการนี้จะใช้สำหรับวัตถุประสงค์ทางวิจัยและการสอน ข้อมูลส่วนตัวต่าง ๆ ของท่านจะถูกเก็บเป็นความลับตามกฎหมาย การเข้าร่วมโครงการวิจัยนี้เป็นไปโดยสมัครใจของท่าน ถ้าท่านตัดสินใจเข้าร่วมท่านมีสิทธิ์ที่จะถอนการยินยอมและหยุดการเข้าร่วมในโครงการนี้เมื่อใดก็ได้ หากท่านต้องการระงับการเข้าร่วมโปรดแจ้ง ทพ.พิศาล สัมปทานุกูล ภาควิชาปริทันตวิทยา คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ชั้น 11 อาคารสมเด็จย่าฯ 93 โทรฯ 02-218-8850

โครงการวิจัยนี้ได้ผ่านการพิจารณาของคณะกรรมการพิจารณาจริยธรรม คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัยแล้ว และผู้กำกับดูแลการวิจัย ผู้ตรวจสอบ และคณะกรรมการพิจารณาจริยธรรมสามารถตรวจสอบขั้นตอนในการวิจัยและข้อมูลอื่น ๆ ได้

เอกสารยินยอมเข้าร่วมการวิจัย (Consent Form)

การวิจัยเรื่อง ผลของอีพิแกลโลคาเทชิน-3-แกลเลทจากชาเขียวต่อกลิ่นปาก

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็น ด้วยเหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

(.....)

ลงนาม.....พยาน

(.....)

ลงนาม.....พยาน

(.....)

ลงนาม.....หัวหน้าโครงการวิจัย

(รศ ดร เอมอร เบญจวงศ์กุลชัย)

วันให้คำยินยอมเข้าร่วมวิจัย วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนาม หรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

(.....)

ลงนาม.....พยาน

(.....)

ลงนาม.....พยาน

(.....)

ลงนาม.....หัวหน้าโครงการวิจัย

(รศ ดร เอมอร เบญจวงศ์กุลชัย)

วันให้คำยินยอมเข้าร่วมวิจัย วันที่.....เดือน.....พ.ศ.....

Appendix: B

เลขที่.....

ใบสมัครเข้าร่วมงานวิจัยเรื่อง

ผลของอีพิแกลโลคาเทชิน แกลเลท ในชาเขียวต่อกลิ่นปาก (การศึกษาในคลินิก)

ชื่อ-นามสกุล.....

เพศ ☐ ชาย ☐ หญิง อายุ..... ปี

เบอร์โทรศัพท์บ้าน..... เบอร์มือถือ..... เบอร์โทรศัพท์ที่ทำงาน.....

กรุณาใส่เครื่องหมาย √ หน้าข้อความที่ตรงกับความเป็นจริงที่สุด

1. ปัจจุบันท่านมีโรคประจำตัว/ภาวะผิดปกติหรือไม่

☐ ไม่มี ☐ มี ดังต่อไปนี้

☐ โรคเบาหวาน ☐ โรคหัวใจ ☐ โรคทางสมอง ☐ โรคไต

☐ โรคตับ ☐ โรคไทรอยด์ ☐ โรคเจ็บป่วยเรื้อรัง

☐ ความดันสูง ☐ อื่นๆ (โปรดระบุ)…….. .

2. ท่านเคยมีโรคประจำตัว/ภาวะผิดปกติหรือไม่

☐ ไม่มี ☐ มี ดังต่อไปนี้

☐ โรคเบาหวาน ☐ โรคหัวใจ ☐ โรคทางสมอง ☐ โรคไต

☐ โรคตับ ☐ โรคไทรอยด์ ☐ โรคเจ็บป่วยเรื้อรัง

☐ ความดันสูง ☐ ผ่าตัดใน 1 ปีที่ผ่านมา

☐ อื่นๆ (โปรดระบุ)…….. .

3. ปัจจุบันท่านได้รับประทานยารักษาโรคอยู่หรือไม่

☐ ไม่ ☐ ทาน (โปรดระบุชื่อยาให้ครบถ้วน)..... . .....

4. ภายใน 1 เดือนที่ผ่านมา ท่านได้รับประทานยาปฏิชีวนะหรือยาฆ่าเชื้อแก้อักเสบหรือไม่

☐ ไม่ ☐ ทาน (โปรดระบุชื่อยาให้ครบถ้วน)..... . .....

(โปรดระบุวันสุดท้ายที่ทานยาเสร็จ).....

5. ท่านมีประวัติการแพ้ยาหรือสารใดๆบ้างหรือไม่

☐ ไม่มี ☐ มี (โปรดระบุ) .....

6. ท่านทานเครื่องดื่มแอลกอฮอล์บ่อยเพียงใด

☐ ไม่ทาน ☐ นานๆครั้ง ☐ บ่อย ☐ ทานประจำ

7. ท่านทานกาแฟบ่อยเพียงใด

☐ ไม่ทาน ☐ นานๆครั้ง ☐ บ่อย ☐ ทานประจำ

8. ท่านทานอาหารที่มีกลิ่นฉุนเช่น ชะอม กระเทียม หัวหอม บ่อยเพียงใด

☐ ไม่ทาน ☐ นานๆครั้ง ☐ บ่อย ☐ ทานประจำ

9. ท่านมีแผลในช่องปากบ่อยเพียงใด ☐ บ่อยๆ ☐ นานๆครั้ง

10. ท่านมีอาการปากแห้งเป็นประจำหรือไม่

☐ ไม่มี ☐ มี

11. ท่านมีการนอนหายใจทางปากหรือไม่

☐ ไม่มี ☐ มี ☐ ไม่ทราบ

12. ท่านเคยมีคนทักว่ามีกลิ่นปากหรือไม่

☐ ไม่เคย ☐ เคย

13.. ท่านสูบบุหรี่หรือไม่

☐ ไม่ ☐ สูบ ☐ เคยสูบแต่เลิกแล้ว (โปรดระบุระยะเวลาที่เลิกสูบ).....

14. ท่านเคยได้รับการรักษาทางปริทันต์ด้วยการผ่าตัดหรือเกลารากฟันหรือไม่

☐ ไม่ทราบ ☐ ไม่เคย ☐ เคย (โปรดระบุวันสุดท้ายที่เสร็จสิ้นการรักษา).....

15. ปัจจุบันท่านใส่ฟันปลอมชนิดถอดได้หรือไม่ ☐ ไม่ ☐ ใส่

16. ท่านแปรงฟันทุกวันหรือไม่ ☐ ใช่ ☐ ไม่ วันละกี่ครั้ง .....

17. ท่านทำความสะอาดช่องปากและฟันด้วยวิธีอะไรเพิ่มเติมบ้าง

☐ใช้แปรงซอกฟัน ☐ ใช้ไหมขัดฟัน ☐ แปรงลิ้น ☐ ใช้ไม้จิ้มฟัน

☐ ใช้น้ำยาบ้วนปาก

.

เลขที่.....

สำหรับคณะวิจัย

ชื่อ-นามสกุล.....

ค่า VSC เบื้องต้น = ……. ppb

จากการตรวจพบว่าเป็น periodontitis ☐ ใช่ ☐ ไม่ใช่

สามารถเป็นอาสาสมัครได้หรือไม่ ☐ ได้ ☐ ไม่ได้

วันที่ทดลอง	ชนิดสาร	ก่อนทดลอง				15 min				30 min				60 min				120 min			
		H	M	D	รวม	H	M	D	รวม	H	M	D	รวม	H	M	D	รวม	H	M	D	รวม

Biography

Mr. Pisal Sampatanukul was born on March 28, 1968 in Bangkok. He graduated in Doctor of Dental Surgery (D.D.S.) from Faculty of Dentistry, Chulalongkorn University in 1992, and became a lecturer of the Department of Oral Radiology, Faculty of Dentistry, Chiangmai University until 1997. After that, he worked as a general denitst at Chamchan Dental Clinic. He was a candidate for a Master of Science Program in Periodontics, Faculty of Dentistry, Chulalongkorn University in 2005.