

Study of Bacteria Associated with Dental Caries Using a 3 Tone Disclosing Agent

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Abstract

The aim of this study was to determine the efficacy of a 3 tone plaque disclosing gel in assessing the risk of caries related to the population of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* quantified using a quantitative real-time polymerase chain reaction (qRT-PCR).

15 healthy children of ages 9 - 12 years were randomly examined. The 3 tone plaque disclosing gel was applied on teeth surfaces, which changed the color to pink or red, blue or purple and light blue. Plaque was divided into 3 groups based on staining. Genomic DNA from each sample was subjected to a qRT-PCR assay for quantitative detection of target bacteria. The Kruskal-Wallis test was conducted for correlation between the color of plaque and the number of bacterial species.

The levels of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.* were significantly different in the plaque samples of the 3 groups ($p < 0.05$). The proportion of *S. sobrinus* to *S. mutans* showed correlation to the color of plaque.

The different color-dyed plaque was related to the number of acidogenic bacteria. The 3 tone plaque disclosing gel could be used as one of the indicators to assess the clinical risk of caries associated with the population of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.*

Key words : 3 Tone disclosing agent, Dental plaque, Quantitative real-time polymerase chain reaction, *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus spp.*

I. Introduction

Dental plaque is the mass of microorganisms that bind on the tooth's surface. It is estimated that more than 700 species of bacteria live in the oral flora[1,2]. These species can exist as part of the dental biofilm. Dental biofilm is composed of about a 10^{12} colony forming unit/ML (CFU/mL)[3]: of which,

Streptococcus mutans, *Streptococcus sobrinus*, and *Lactobacillus spp.* are significantly associated with the development and progression of dental caries.

In the dental biofilm, determining the number of *S. mutans* and *S. sobrinus* is shown to be the method of predicting caries activity[4,5]. *S. mutans* is more frequently detected than *S. sobrinus* in the oral cavity[6]. However, several studies have

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shown that *S. sobrinus* was more closely associated than *S. mutans* with high caries activity in children[7]. Children with early childhood caries (ECC) had higher levels of *S. mutans* and *S. sobrinus* than children with caries-free dental plaque samples: moreover, children with higher incidence of ECC showed a high ratio of *S. sobrinus* to *S. mutans* in their dental plaque samples[8].

While *S. mutans* and *S. sobrinus* are significantly linked with the development of dental caries, *Lactobacillus spp.* are significantly linked with its progression[9]. *Lactobacillus spp.* have the ability to generate and survive in a low pH environment[10,11]. Moreover, they were reportedly higher in active caries lesions than in arrested caries lesions: and decrease in the level of pH in caries lesions was associated with an increased prevalence of *Lactobacillus spp.*[12]. The growth of *Lactobacillus spp.* was more rapid than that of *S. mutans* at low pH[13,14].

Several studies on the methods of risk-assessment for caries have been conducted in the field of cariology. In addition, several methods have been used to detect *S. mutans* and identify the pathogenicity of biofilm. However, most of these methods are relatively non-specific, time-consuming, and laborious.

Recently, a 3 tone plaque disclosing gel (GC Tri Plaque ID Gel™: GC corporation, Tokyo, Japan) was developed based on the correlation between caries risks and production of acids from dental plaque in response to sucrose. Although conventional plaque disclosing gel is cost-effective and can be used easily in real-time chairside, it lacks the ability for assessment of plaque ecology such as acid production by fermentation. The 3 tone plaque disclosing gel was developed to aid patient oral hygiene education. It contains different dyes (Rose bengal, Coomassie Brilliant blue, Brilliant Blue FCF) and sucrose. It indicates both dental plaque fermentation and plaque acid production by color within 2 min.

In newly-formed plaque, the biofilm is sparse. Blue pigments are easily washed off because of low molecular weight and red pigments remain because of high molecular weight. So, new plaque is dyed pink or red color. As the biofilm matures (> 48 hours), its structure becomes dense, both red and blue pigments are trapped, and the old plaque is dyed blue or purple in color. In cases with high risk of plaque, the sucrose in the 3 tone plaque disclosing gel is metabolized by acidogenic bacteria in the biofilm. As bacteria produce acid, the plaque pH drops to pH 4.5, which results in the disappearance of red pigments and appearance of a light blue color[15].

In a previous study using the 3 tone plaque disclosing gel,

Jayanthi M *et al.*[15] confirmed the relationship between caries, plaque, and cariogenic microorganisms. As children developed mature and pathological plaque, the number of *S. mutans* in plaque was increased. This result showed the efficacy of the 3 tone plaque disclosing gel in identifying the risk of caries associated with plaque. However, the relationship between caries progression and cariogenic bacteria was not reported by these authors, since *Lactobacillus spp.* which is responsible for caries progression was not included in their study.

The aim of this study was to assess the efficacy of the 3 tone plaque disclosing gel to assess the clinical risk of caries associated with the population of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.* quantified using a quantitative real-time polymerase chain reaction (qRT-PCR).

II. Materials and methods

The Institutional Review Board (IRB) of Gangneung-Wonju National University dental hospital (IRB No.: 2015-016) approved the study prior to its implementation. A single operator performed all the experimental procedures.

1. Subjects and plaque sampling

15 healthy children aged 9 - 12 years old who visited Gangneung-Wonju National University dental hospital were randomly examined. Children who had not used mouth rinses or any medication during past 3 months and without any prosthetic or orthodontic appliances which modify surface characteristics were included. The children willingly participated in this study and informed parental consent was obtained.

The 3 tone plaque disclosing gel was applied on all tooth surfaces with a microbrush (Fig. 1). The compositions of the 3 tone plaque disclosing gel used in this study are presented in Table 1. Dyed tooth-surfaces were left undisturbed for 2 min. Subsequently, the tooth-surfaces were changed to pink or red, blue or purple, and light blue (Fig. 2). The tooth-surfaces were gently washed using a 3-way syringe for 30 sec. In all 15 children who participated in the experiment, the plaque was stained with 3 tone dyed colors (pink or red, blue or purple, light blue).

Depending on the colors, the plaque were divided randomly into 3 experimental groups. Group I was pink or red plaque. Group II was blue or purple plaque. Group III was light blue plaque (Table 2).



Fig. 1. The 3 tone plaque disclosing gel was applied on tooth surfaces with a microbrush.



Fig. 2. The tooth-surfaces were changed to pink or red, blue or purple, and light blue.

Table 1. Composition of the 3 tone plaque disclosing gel used in the study

Disclosing gel		Composition	Manufacturer
GC Tri Plaque ID Gel™	Paste	Glycerin, Water, Potassium sorbate, Flavoring oil, Saccharin, Citric acid, Sucrose	GC corporation, Tokyo, Japan
	Dyes	Rose bengal, Coomassie Brilliant Blue, Brilliant Blue FCF	

Table 2. Experimental and group classification

Group	Plaque color
I (n = 15)	Pink or red
II (n = 15)	Blue or purple
III (n = 15)	Light blue

The 3 tone dyed plaque was separately collected from the sound tooth surfaces with a sterile spoon excavator. Only plaque with clearly dyed color was collected. The collection was conducted by a sweeping action and all collected plaque was placed on the supragingiva. Samples were collected from one of the available teeth: sites based on the plaque abundance, maxillary right first molar, mandibular left molar, maxillary incisor or mandibular incisor. The collection sites were chosen from the first preference site to the next available preferred site. To collect the same amount of plaque, plaque was stored in a sterile acrylic resin block (Jet Tooth Shade™ Powder: Lang Dental Mfg Inc, Wheeling) with a hole that was made by #2 round bur (RA 2 Stainless steel bur: Komet, Germany) pen-

etrating to a 1 mm depth. After collection of the plaque, the extra plaque was cut at the level of the acrylic resin block surface. The acrylic resin blocks with plaque were kept in sterile 1.7 mL tube containing 1 mL sterile distilled water. The samples were stored at -20°C until extraction of bacterial DNAs.

2. Extraction of DNA

Genomic DNA Extraction Kit (iNtRON Biotechnology Inc., Sungnam, Korea) was used to extract and purify bacterial DNAs from each plaque sample. DNAs were extracted according to the manufacturer's procedures. Briefly, plaque sample and acrylic resin block was stored at -20°C sterile in 1.7 mL tube containing 1 mL sterile distilled water. The mixture was mixed vigorously by vortex to separate the plaque from acrylic resin block and acrylic resin block was taken out. The mixture was centrifuged at 13000 rpm for 30 sec to remove supernatant. To lyse cells, 50 µL of pre-buffer and 3 µL of lysozyme were added to the tube and mixed well. The mixture was incubated at 37°C for 15 min and mixed well by pipetting 3 times every 5 min. Then, 250 µL of G-buffer solution was

added and mixed gently by pipetting at least 10 times. This step conducted to pass efficiently cell lysates through a column and increase gDNA binding onto column resins for efficient deproteinization. The sample was applied to G-spin column and centrifuged at 13,000 rpm for 1 min. After centrifugation, the collection tube was removed from column and filtrate was discarded. To wash, 500 μ L of washing buffer A was added to column. The column was centrifuged at 13,000 rpm for 1 min. After centrifugation, the collection tube was removed from column and filtrate was discarded. The column was placed back in the same collection tube. 500 μ L of washing buffer B was added to column. The column was centrifuged at 13,000 rpm for 1 min. The column was centrifuged at 13,000 rpm for an additional 1 min to dry the filter membrane. The column was placed into a 1.5 mL micro centrifuge tube. To elute DNA, 100 μ L elution buffer was added to the center of the column. The column was incubated at room temperature for 1 min and centrifuged at 13,000 rpm for 1 min. The extracted DNAs were stored at -20°C until use.

3. Quantitative real-time PCR

The numbers of colony forming unit (CFU) were obtained by plating culture dilutions on Brain heart infusion (BHI) agar plates (Difco Laboratories, Detroit, USA)[16,17]. After confirming the number of CFU (10^8 CFU/mL), serial dilutions of the *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* suspension were produced.

Streptococcus mutans ATCC 25175, *Streptococcus sobrinus*

B-13, and *Lactobacilli acidophilus*. ATCC 4355 were the bacterial strains used for the standard curve values of each respective strain. The sequences of primers used are listed in Table 3[18-20]. Genomic DNA from each sample was subjected a quantitative real-time polymerase chain reaction (qRT-PCR) assay using the ABI PRISM 7500 system (Applied Biosystems, California, USA) for quantitative detection of target bacteria: Oral bacteria, *S. mutans*, *S. sobrinus* and *Lactobacillus spp.*

Each reaction contained a total of 20 μ L of mixture, which included 10 μ L of Power SYBR Green PCR master mix (TOPreal™ qPCR 2X PreMIX: Enzynomics, Daejeon, Korea), 1 μ L of DNA extracted from samples, 1 μ L of each of the two primers (10 pmol/ μ L) and 7 μ L of nuclease-free water. The temperature was as follows: 10 min at 95°C for activation, denaturation followed by 40 cycles for 15 sec at 95°C, primer annealing and extension for 1 min at 64°C for *S. mutans* and *S. sobrinus* as well as at 62°C for *Lactobacillus spp.* Data acquisition and subsequent analysis were performed using 7500 Software version 2.0.6 (Applied Biosystems, California, USA). Purification of detectable genomic DNA from all plaque samples was confirmed by universal primers.

4. Statistical analysis

Statistical evaluation was performed using the Kruskal-Wallis test with SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at a 0.05 probability level. The Mann-Whitney test was performed for post hoc comparisons of the groups.

Table 3. Primers used in this study

Bacteria	Primer	Sequence (5'-3')	Position (5')	References
Universal primer	Ust1	GAACGGGTGAGTAACGCGTAGGT	106	Morita <i>et al.</i> [18]
	Ust2	CACTCACGCGCGTTGCTCGGTC	387	
<i>S. mutans</i>	Sm F5	AGCCATGCGCAATCAACAGGTT	2007	Yano <i>et al.</i> [19]
	Sm R4	CGCAACGCGAACATCTTGATCAG	2421	
<i>S. sobrinus</i>	Ss F3	GAAACCAACCCAACCTTAGCTTGGAT	2123	Yano <i>et al.</i> [19]
	Ss R1	ATGGAGTGATTTCCATCGGTACTTG	2451	
<i>Lactobacillus spp.</i>	LactoF	TGGAAACAGRTGCTAATACCG	157	Byun <i>et al.</i> [20]
	LactoR	GTCCATTGTGGAAGATTCCC	379	

S. mutans = *Streptococcus mutans*, *S. sobrinus* = *Streptococcus sobrinus*

III. Results

To determine the linearity and bacterial detection, serially diluted genomic DNA solutions of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* were amplified by quantitative real-time PCR (Fig. 3).

The critical threshold cycle (C_T) mean is the cycle at which the fluorescence becomes detectable beyond background. C_T is negatively proportional to the logarithm of the initial number of colony forming unit (CFU). The standard curves for each of the bacteria were plotted for each primer set by using C_T

values obtained from the amplification of genomic DNA extracted.

The number of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.* in plaque samples of 15 children were determined by conversion of the C_T (Table 4). *S. mutans*, *S. sobrinus* and *Lactobacillus spp.* were detected in all plaque samples .

The number of *S. mutans* was significant different in pink or red, blue or purple, light blue plaque samples ($p < 0.05$). Group I ($3.6 \times 10^4 \pm 4.8 \times 10^4$) showed the smallest value, followed by group II ($1.9 \times 10^5 \pm 2.8 \times 10^5$), and group III ($3.2 \times 10^5 \pm 4.6 \times 10^5$) had the largest value (Table 4).

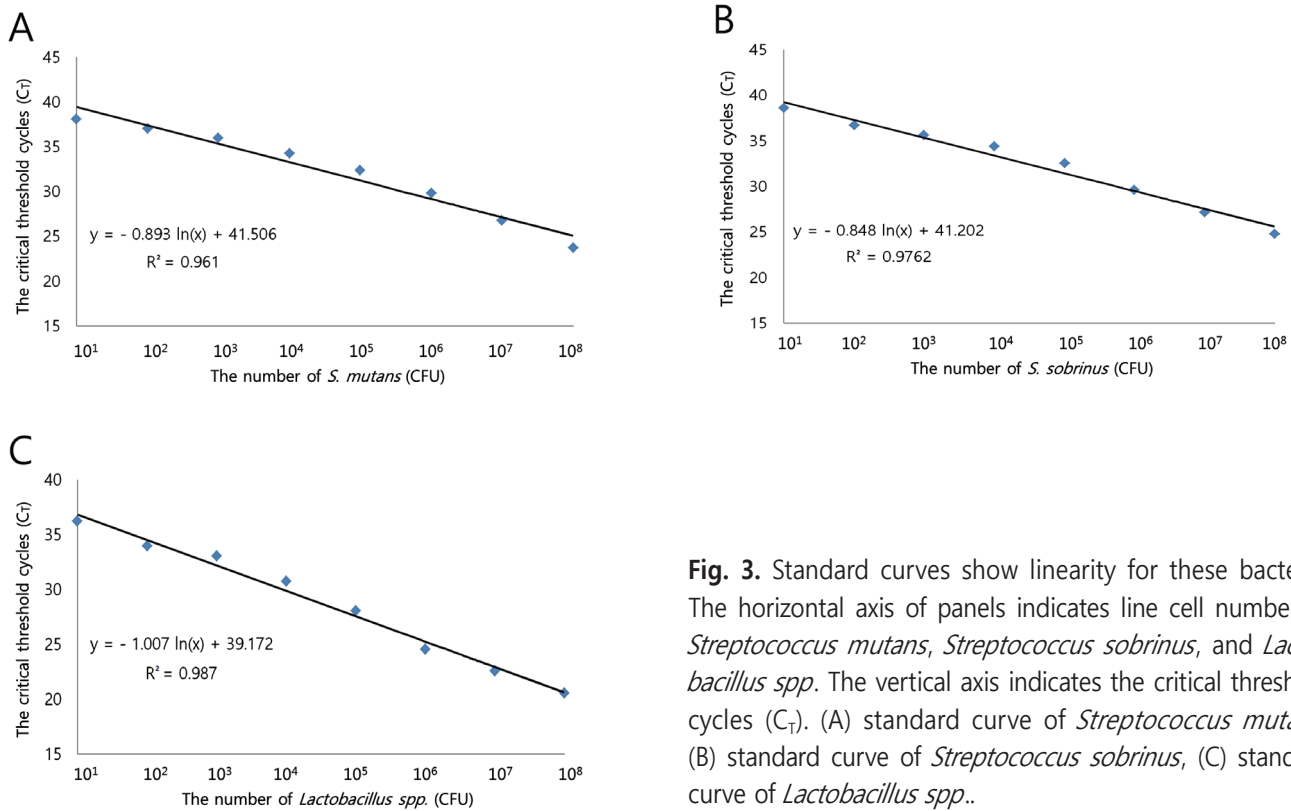


Fig. 3. Standard curves show linearity for these bacteria. The horizontal axis of panels indicates line cell number of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* The vertical axis indicates the critical threshold cycles (C_T). (A) standard curve of *Streptococcus mutans*, (B) standard curve of *Streptococcus sobrinus*, (C) standard curve of *Lactobacillus spp.*.

Table 4. Number of colony forming unit in plaque sample

Group	Oral bacteria	Mean \pm SD (Number of CFU)		
		<i>Streptococcus mutans</i>	<i>Streptococcus sobrinus</i>	<i>Lactobacillus spp.</i>
I (n = 15)	$5.5 \times 10^7 \pm 7.8 \times 10^6$	$3.6 \times 10^4 \pm 4.8 \times 10^4$ ^a	$1.5 \times 10^2 \pm 1.5 \times 10^2$ ^d	$1.7 \times 10^5 \pm 3.2 \times 10^5$ ^g
II (n = 15)	$5.7 \times 10^7 \pm 8.1 \times 10^6$	$1.9 \times 10^5 \pm 2.8 \times 10^5$ ^b	$9.6 \times 10^2 \pm 1.0 \times 10^3$ ^e	$7.8 \times 10^5 \pm 9.5 \times 10^4$ ^h
III (n = 15)	$5.7 \times 10^7 \pm 7.2 \times 10^6$	$3.2 \times 10^5 \pm 4.6 \times 10^5$ ^c	$2.0 \times 10^3 \pm 1.8 \times 10^3$ ^f	$2.7 \times 10^6 \pm 1.7 \times 10^6$ ⁱ

CFU = colony forming unit, SD = standard deviation

^{a, b, c, d, e, f, g, h, i}: Different letters denote significantly differences between groups ($p < 0.05$, Kruskal-Wallis test)

The number of *S. sobrinus* was also significantly different among pink or red, blue or purple, light blue plaque samples, respectively ($p < 0.05$). Group I ($1.5 \times 10^2 \pm 1.5 \times 10^2$) showed a lower value than group II ($9.6 \times 10^2 \pm 1.0 \times 10^3$), and group III ($2.0 \times 10^3 \pm 1.8 \times 10^3$) had showed the highest among the 3 groups (Table 4).

The number of *Lactobacillus spp.* showed significant difference in pink or red, blue or purple, light blue plaque sample, respectively ($p < 0.05$). The counts of group I ($1.7 \times 10^5 \pm 3.2 \times 10^5$) were significantly lower than group II ($7.8 \times 10^5 \pm 9.5 \times 10^4$), and group III ($2.7 \times 10^6 \pm 1.7 \times 10^6$) had the highest counts among the 3 groups (Table 4).

In the same color of plaque sample, the number of *S. sobrinus* showed the smallest value, followed by *S. mutans*, and *Lactobacillus spp.* had the largest value.

Ratio of *S. sobrinus* to *S. mutans* in dental plaque sample was different in pink or red, blue or purple, light blue plaque sample, respectively. Group I (0.4×10^{-2}) showed a lower value than group II (0.5×10^{-2}), and group III (0.6×10^{-2}) showed the highest value among the 3 groups ($p = 0.17$) (Table 5).

Ratio of *S. mutans* to oral bacteria in dental plaque sample was increased based on the color of plaque from group I to group III. Group I (0.65×10^{-3}) showed the smallest value, followed by group II (0.33×10^{-2}), and group III (0.56×10^{-2}) had the largest value ($p = 0.18$) (Table 5).

Level of *S. sobrinus* to oral bacteria in dental plaque sample was different based on the color of plaque. Group I (0.27×10^{-5}) showed a lower value than group II (1.68×10^{-5}), and group III (0.35×10^{-4}) showed the highest value among the 3 groups ($p = 0.20$) (Table 5).

Level of *Lactobacillus spp.* to oral bacteria in dental plaque sample was increased based on the color of plaque from group I to group III. The number of Group I (0.30×10^{-2}) showed a lower than group II (1.36×10^{-2}). And group III (0.47×10^{-1}) had the highest number among the 3 groups ($p = 0.29$) (Table 5).

IV. Discussion

In the present study, as dental plaque became more fermented, the number of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* was increased based on the color of plaque from group I to group III. Group I (pink or red) showed the smallest value, followed by group II (blue or purple), and group III (light blue) had the largest value in the number of *S. mutans*, *S. sobrinus* and *Lactobacillus spp.* And ratio of *S. mutans*, *S. sobrinus*, *Lactobacillus spp.* to oral bacteria was increased based on the color of plaque from group I to group III. This finding confirmed that the different color-dyed plaque reflect the pathogenicity associated with cariogenic bacteria.

Recent research on the maturity and the change of bacterial species in biofilm formation indicates that the dental biofilms produce acids from carbohydrates which results in caries formation. Under severe and prolonged acidic conditions, more aciduric bacteria such as *S. mutans* and *Lactobacillus spp.* become dominant[21]. Similarly, Duchin *et al.*[22] showed that plaque from the caries lesions showed markedly elevated levels of *S. mutans*, as compared to plaque from sound enamel. Boyar and Bowden *et al.*[23,24] demonstrated that higher lev-

Table 5. Ratio of colony forming unit in plaque sample

Group	Mean \pm SD (Number of CFU)			
	Sm / Oral bacteria	Ss / Oral bacteria	Lacto / Oral bacteria	Ss / Sm
I (n = 15)	0.65×10^{-3}	0.27×10^{-5}	0.30×10^{-2}	0.4×10^{-2}
II (n = 15)	0.33×10^{-2}	1.68×10^{-5}	1.36×10^{-2}	0.5×10^{-2}
III (n = 15)	0.56×10^{-2}	0.35×10^{-4}	0.47×10^{-1}	0.6×10^{-2}
p value	0.18	0.20	0.29	0.17

Kruskal-Wallis test (*: $p < 0.05$)

CFU = colony forming unit, SD = standard deviation

Sm = *Streptococcus mutans*, Ss = *Streptococcus sobrinus*, Lacto = *Lactobacillus spp.*

Ss / Sm = A ratio of *S. sobrinus* to *S. mutans*

els of *S. mutans* and *Lactobacillus spp.* occurred at sites where caries developed. Igrashi *et al.*[25] concluded that enhanced production of acid observed in the plaque from white spot lesions reflected the presence of highly acidogenic cariogenic bacteria such as *S. mutans* and *Lactobacillus spp.* Based on these reports, the development and progression of caries is associated with the number of cariogenic bacteria such as *S. mutans* and *Lactobacillus spp.*

The previous study by Jayanthi M *et al.*[15] had limitation to assess dental caries progression, since *Lactobacillus spp.*, which is responsible for the progression of caries, was not included. To overcome the limitations of previous study, *Lactobacillus spp.* was included in the experimental group. In this study, we showed that as dental plaque became more fermentated, the number of *Lactobacillus spp.* was increased. Thus, 3 tone disclosing gel can be used to assess the risk of dental caries progression.

Clinically, the number of acidogenic bacteria shows highest correlation with children's dmfs (decayed, missing, filled smooth surface of tooth). Choi EJ *et al.*[8] reported that children with early childhood caries (ECC) had a significantly higher level of *S. mutans* in their dental plaque samples. Although *S. sobrinus* was detected less frequently and in lower numbers than *S. mutans*, the ratio of *S. sobrinus* to *S. mutans* showed highest correlation with children's dmfs. In our study, as dental plaque became more fermentated, the proportion of *S. sobrinus* to *S. mutans* was increased. To examine the relation of these bacteria to caries prevalence and the DMFT index, we should have surveyed the data about the participant's DMFT count and caries prevalence. Additional supplementation is needed. Group I (pink or red) showed the smallest value, followed by group II (blue or purple), and group III (light blue) had the largest value. The results suggested that a light blue stained plaque is linked with a high possibility of ECC.

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the population of acidogenic bacteria, because it is the most reliable and accurate tool to determine bacterial concentrations in clinical samples.

With qRT-PCR, a broad range of bacterial cells can be measured. The specificity and sensitivity of detection can be provided by specific primers amplifying species-specific target sequence in the PCR[18-20].

A number of clinical chairside tools have been developed to assess the caries risk such as saliva check (Saliva-check mutans: GC corporation, Tokyo, Japan), plaque check (Plaque-check+pH

diagnostic kit: GC corporation, Tokyo, Japan), and pH meter (ISFET micro-pH sensor: Horiba, Kyoto, Japan). They are easily accessible but, commercial kits are still expensive. The 3 tone disclosing gel could be used at chair-side, within a clinically acceptable time and at low cost.

Alaluusua and Roeters *et al.*[26,27] reported that visible plaque of maxillary incisors was strongly associated with the development of caries, and the best indicator of caries risk was visible plaque compared to other potential indicators. Wendt *et al.*[28] reported that children with no visible plaque at 2 years old had greater chances of remaining free of caries until 3 years old, as compared to children with visible plaque.

Clinically, Real-time caries activity diagnosis at chairside is important to dentists. The results of the present study confirmed the relationship between caries risk, plaque, and cariogenic microorganisms. As per color of plaque, the increasing pathological bacteria counts showed. The 3 tone plaque disclosing gel could be used as one of the indicators to assess the clinical risk of caries associated with the population of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.* The use of the 3 tone disclosing gel could promote patient education and their motivation for better oral care.

V. Conclusion

The relationship between the different color-dyed plaque and the number of acidogenic bacteria was confirmed. The 3 tone plaque disclosing gel could be used as one of the indicators to assess the clinical risk of caries associated with the population of *S. mutans*, *S. sobrinus*, and *Lactobacillus spp.* The use of the 3 tone disclosing gel could promote patient education and their motivation for better oral care.

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국문초록

세가지 색상차이를 보이는 착색제를 이용한 치아 우식 관련 균에 관한 연구

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본 연구는 치태의 성숙도에 따라 치태를 서로 다른 색상으로 염색하는 GC Tri Plaque ID Gel™(GC corporation, Tokyo, Japan)을 이용하여 치아 우식 위험도를 평가하고자 하였다. 치아 우식의 발생 및 진행과 연관된 균인 *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus spp.*의 수를 Quantitative real-time polymerase chain reaction (qRT-PCR)로 측정하여 치아 우식 위험도를 보았다.

본 실험은 강릉원주대학교 치과병원 임상시험 심사위원회의 심의를 받고 진행하였다. 강릉원주대학교 치과병원 소아치과에 내원한 전신질환이 없는 건강한 9 - 12세의 초등학생 15명의 치면을 착색제로 염색하였다. 치태의 성숙도에 따라 서로 다른 세가지 색상으로 염색되었으며 색상별로 3개의 실험군인 I군(pink/red), II군(blue/purple), III군(light blue)으로 나누었다. 3개의 실험군에서 각각 DNA를 추출한 후, qRT-PCR을 이용하여 *S. mutans*, *S. sobrinus*, *Lactobacillus spp.*의 수를 측정하였다.

3개의 실험군 사이에 *S. mutans*, *S. sobrinus*와 *Lactobacillus spp.* 균 수의 유의한 차이가 관찰되었으며 3종류의 균 모두 III군에서 가장 많이 관찰되었다($p < 0.05$).

GC Tri Plaque ID Gel™는 기존 착색제와는 달리 치태의 성숙도에 따라 서로 다른 세가지 색상으로 염색되며, 치태 염색 색상의 차이는 치아 우식 관련 균 수의 차이를 보여주었다. GC Tri Plaque ID Gel™이 치아 우식 위험도를 평가하는 하나의 지표로서 사용될 수 있는 가능성을 확인하였다.