

## An Analysis of the Periodontopathic Bacteria Involved in Extrinsic Discoloration of Teeth

Kie Matsuda<sup>1</sup>, Natsuki Suwa<sup>2</sup>, Hitomi Kikuchi<sup>3</sup>, Tsuneo Sekimoto<sup>1</sup> and Akane Imai<sup>3,4\*</sup>

<sup>1</sup>Department of Pediatric Dentistry, School of Life Dentistry at Niigata, The Nippon Dental University, Niigata, Japan

<sup>2</sup>NIAD-UE-Recognized Advanced Course, College at Niigata, The Nippon Dental University, Niigata, Japan

<sup>3</sup>Department of Dental Hygiene, College at Niigata, The Nippon Dental University, Niigata, Japan

<sup>4</sup>Department of Biochemistry, School of Life Dentistry at Niigata, The Nippon Dental University, Niigata, Japan

**\*Corresponding author:** Akane Imai, PhD, Professor, Department of Dental Hygiene, College at Niigata, The Nippon Dental University, Niigata, Japan; Tel: +81 25 267 1500; Fax: +81 25 267 1510; E mail: imaiak@ngt.ndu.ac.jp

**Article Type:** Research, **Submission Date:** 22 June 2016, **Accepted Date:** 18 July 2016, **Published Date:** 01 August 2016.

**Citation:** Kie Matsuda, Natsuki Suwa, Hitomi Kikuchi, Tsuneo Sekimoto and Akane Imai (2016) An Analysis of the Periodontopathic Bacteria Involved in Extrinsic Discoloration of Teeth. J. Dent. Sci. Ther 1(2): 1-4.

**Copyright:** © 2016 Kie Matsuda, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

Recent dental esthetics are paying attention to whitening. Many people take an interest in tooth color, and wish to remove extrinsic discoloration. While a relationship between tooth discoloration and periodontopathic bacterial prevalence has been suggested, such a relationship has not been clearly elucidated. The aim of the present study was to clarify the relationship between the periodontopathic bacteria present in plaque and tooth discoloration. Plaque samples were taken from 54 subjects whose tooth color was evaluated using a shade guide. The subjects were then divided into a white group (30 women) and a discolored group (24 women). Using bacterial DNA prepared from the plaque samples as a template, polymerase chain reaction was performed with specific primers to identify the presence of five periodontopathic bacteria (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola* and *Prevotella intermedia*). The numbers of the bacterial species were counted and the percentage of the subjects that were carriers of each bacterium was calculated. In addition, the mean number ( $\pm$  SE) of periodontopathic bacterial species per person was calculated as 1.23 ( $\pm$  0.15) and 2.04 ( $\pm$  0.21) in the white and discolored groups, respectively, and there was a significant difference between these two groups ( $p < 0.01$ ). We thus found a positive relationship between superficial tooth color and the number of periodontopathic bacterial species.

**Keywords:** Teeth color, Extrinsic discoloration, Shade guide, Periodontopathic bacterial species, Polymerase chain reaction.

### Introduction

The cause of tooth discoloration can be intrinsic or extrinsic [1]. Intrinsic tooth discoloration is the result of aging, exposure to tetracycline antibiotics, excessive fluoride intake during the enamel formation period, or other factors. Extrinsic discoloration is known to be the result of various factors including food and drink, salivary components, smoking, and bacteria. While a relationship between tooth discoloration and periodontopathic

bacterial prevalence has been suggested, its exact mechanism has not yet been well clarified. In this study, we focused on the relationship between tooth discoloration and periodontopathic bacteria by examining five types of periodontopathic bacteria involved in the formation of biofilm [2,3].

The aim of the present study was to clarify the relationship between the periodontopathic bacteria present within intraoral plaque and tooth discoloration by investigating tooth color and periodontopathic bacterial prevalence in healthy, young adult women. On the basis of this examination, the relationship found between tooth color and bacterial prevalence was reported here.

### Material and methods

The subjects were 54 students of the Nippon Dental University College at Niigata (aged 18–22 years, mean age: 18.98  $\pm$  1.11 years, all female and healthy). This study was conducted following review and approval by the ethical review board of the Nippon Dental University, College at Niigata (NDUC-58) in full accordance with ethical principles including the World Medical Association Declaration of Helsinki. The subjects provided written informed consent and agreed to provide plaque samples and to be examined for intraoral findings (tooth coloration). Tooth color was measured in the present study using a composite resin shade guide (Clearfil AP-X Accessory, Kuraray Noritake Dental Inc., Tokyo, Japan) (Figure 1), which is a convenient method for tooth color matching [4]. A typical shade guide was used in the present study as a screening test to measure tooth color. The teeth color of the subjects were estimated by the shade guides (Figure 1). Of the shades shown, “X1” indicates clear white; shades A1 to A3 are reddish-browns where the color darkens in the order of A1 to A2 to A3; shades B2 and B3 are reddish-yellows, where B3 is a darker shade than B2. Shades may differ slightly depending on the manufacturer and the same manufacturer may adopt a variety of composite resin systems. These shade guides are widely available and easy to use, formed essentially of a layer of ivory porcelain and a layer of enamel porcelain. Thus, it is difficult



Figure 1: Tooth shade guides were used in this investigation

to select natural teeth color using [5,6]. For accurate shade evaluation, discolored teeth and natural teeth need to be analyzed using precise equipment [5,6]. However, in the present study we used a shade guide because of the large subject sample and the simplicity of the tooth color evaluation. We also considered that we should clarify whether tooth discoloration is related to the prevalence of periodontopathic bacteria by roughly dividing subjects into white and discolored tooth groups based primarily on tooth brightness.

Next, to estimate the percentage of carriers for each periodontopathic bacteria, we investigated periodontopathic bacterial species in the dental plaques of all subjects using polymerase chain reaction (PCR) of periodontopathic bacterial DNA prepared from sampled plaques of the subjects, with primers specific to the five bacteria [*Aggregatibacter actinomycetemcomitans* (*A. a.*), *Porphyromonas gingivalis* (*P. g.*), *Tannerella forsythensis* (*T. f.*), *Treponema denticola* (*T. d.*) and *Prevotella intermedia* (*P. i.*) (Table 1)]. Plaque sampling and DNA extraction were performed in accordance with the methods described by Shimomura-Kuroki *et al* [7,8]. The DNA templates were prepared from the plaque samples, and used for the PCR with PCR SuperMix (Invitrogen, Carisbad, CA, USA) using specific primers to each of the five periodontopathic bacteria (Table 1) to identify the periodontopathic bacteria in plaque [7,8]. PCR conditions were as previously described [7,8]. To identify the periodontopathic bacteria in each plaque sample, the DNA size of the PCR product was confirmed using 2% agarose gel electrophoresis. Positive control PCR using the DNA of each periodontopathic bacterium (provided by Dr. Mikami M.) was performed, and provided each PCR product size, as shown in Figure 2.

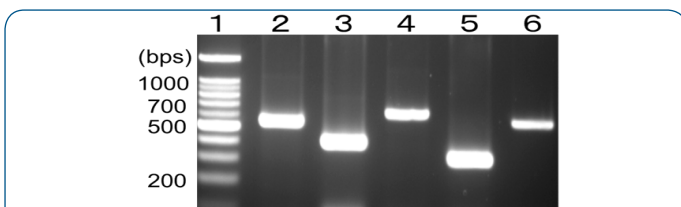


Figure 2: Agarose-electrophoresis of polymerase chain reaction (PCR) products from periodontopathic bacteria. Single DNA bands of the predicted size were obtained by PCR using specific primers as described in Materials and methods. Lane 1, 100 base pair (bp) DNA ladder; lane 2, *Aggregatibacter actinomycetemcomitans* (*A. a.*), 557 bp; lane 3, *Poryphyomonas gingivalis* (*P. g.*), 404 bp; lane 4, *Tannerella forsythia* (*T. f.*), 641 bp; lane 5, *Treponema denticola* (*T. d.*), 316 bp; lane 6, *Prevotella intermedia* (*P. i.*), 575 bp. Molecular weights are shown on the left

Table 1: Species-specific primers for the detection of bacteria by PCR

Primer pairs (5'-3')	Base Position (bp)
<b><i>Aggregatibacter actinomycetemcomitans</i> (<i>A. a.</i>)</b> AAA CCC ATC TCT GAG TTC TTC TTC ATG CCA ACT TGA CGT TAA AT	478-1,034 (557)
<b><i>Porphyromonas gingivalis</i> (<i>P. g.</i>)</b> AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	729-1,132 (404)
<b><i>Tannerella forsythensis</i> (<i>T. f.</i>)</b> GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	120-760 (641)
<b><i>Treponema denticola</i> (<i>T. d.</i>)</b> TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	193-508 (316)
<b><i>Prevotella intermedia</i> (<i>P. i.</i>)</b> TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	458-1,032 (575)

PCR, polymerase chain reaction

## Results and Discussion

The subject numbers of the teeth color based on the shade guides are shown in Figure 3. The subjects were thus divided accordingly into a white group (XL, A1, and A2; n = 30) and a discolored group (A3, B2, and B3; n = 24), which were then used for analysis.

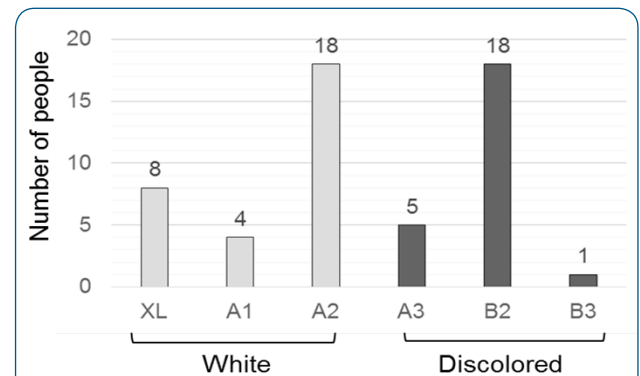
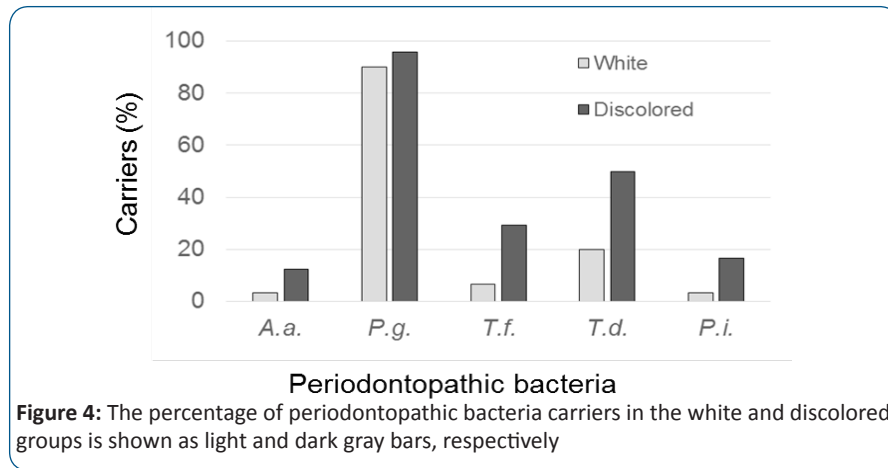


Figure 3: The number of subjects grouped by tooth color according to the shade guide. The subjects were divided into white (30 women) and discolored (24 women) groups

The percentages of the 54 subjects that were carriers for each bacterium were as follows: *P. g.*, 92.6% (50 carriers); *T. d.*, 29.5% (16 carriers); *T. f.*, 16.7% (9 carriers); *P. i.*, 9.3% (5 carriers); and *A. a.*, 7.4% (4 carriers), as shown in Table 2. Fifty subjects (92.6%) had at least one type of periodontopathic bacterium, while four subjects had none of the five analyzed periodontopathic bacteria. These four subjects were therefore not *P. g.* carriers. In addition, to investigate a relationship between tooth color and the presence of periodontopathic bacteria, the percentage of carriers of each bacterium was calculated for each of the white and the discolored groups (Figure 4). The percentage of *P. g.* carriers in the white group was more than 90%, and was similar to that of the discolored group. However, the prevalence of four other bacteria (*A. a.*, *T. f.*, *T. d.* and *P. i.*) in the discolored group

**Table 2:** Counts of teeth color-dependent carriers

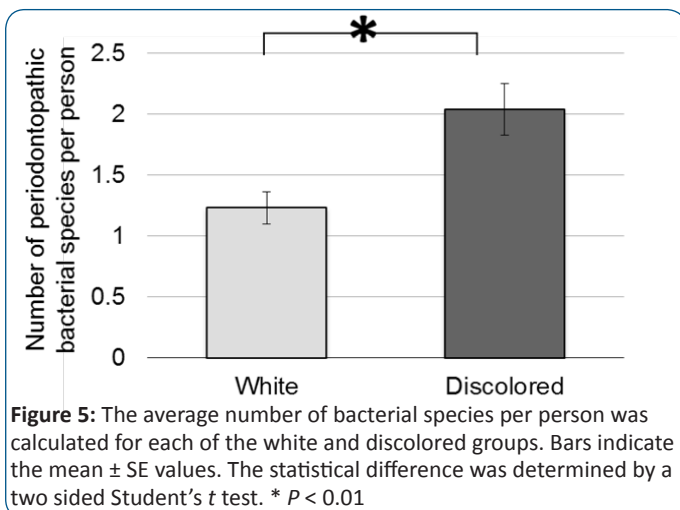
Teeth color	White group			discolored group			total	%
	XL	A1	A2	A3	B2	B3		
Carriers <i>A. a.</i>	0	0	1	2	1	0	4	7.4
<i>P. g.</i>	8	4	15	5	17	1	50	92.6
<i>T. f.</i>	0	0	1	1	6	1	9	16.7
<i>T. d.</i>	2	2	2	2	7	1	16	29.6
<i>P. i.</i>	0	0	1	1	3	0	5	9.3
Total	8	4	18	5	18	1		



**Figure 4:** The percentage of periodontopathic bacteria carriers in the white and discolored groups is shown as light and dark gray bars, respectively

was approximately 2-5 times that in the white group, as shown in Figure 4. These results suggested an absolute requirement for the presence of other periodontopathic bacteria in subjects for teeth discoloration. Finally, we calculated the average number of periodontopathic bacterial species per person (*p.p.*) in the two color groups. The mean ( $\pm$  SE) value of the white group was 1.23 ( $\pm$  0.13) types *p.p.* and that of the discolored group was 2.04 ( $\pm$  0.21) types *p.p.* A *t*-test that was used to compare the white and discolored groups revealed a significant difference ( $p < 0.01$ ) between them (Figure 5). The finding that there was a significantly greater number of species in the discolored compared to the white group, suggested that tooth discoloration and the number of different periodontopathic bacterial species present are related. Figure 4 clearly showed that the prevalence of four bacteria (*A. a.*, *T. f.*, *T. d.* and *P. i.*) in the discolored group

was more than that in the white group. In particular, XL and A1 subjects in the white group was never the carriers of *A. a.*, *T. f.* and *P. i.*, as shown in Table 2. This result suggested *A. a.*, *T. f.* and *P. i.* may induce the discoloration. However, tooth discoloration is the result of not only of bacteria but also of various other known factors including food, drink, and salivary components. In the present study, for example, four subjects were found to have *A. a.*, three of whom were in the discolored group and presented with tea stains, which are caused by components of tea or foods (data not shown). Black stain, which is a form of bacterial discoloration, is reportedly caused by iron ions and *A. a.*, which, as mentioned above, is a type of periodontopathic bacterium [2]. The results of the present study also indicated the possibility that *A. a.* is involved in food-related discoloration of teeth. It is likely that teeth discoloration is caused by *T. f.*, *T. d.* and *P. i.* together with other factors, including other bacterial species. Further investigation is necessary to clarify the cause-and-effect relation of many factors such as food, drink, salivary components, and periodontopathic bacteria to teeth discoloration. A previous report of mechanical tooth cleaning and other procedures in young adults with mild periodontopathic bacterium-related gingivitis or normal gingiva found that intraoral test values and periodontopathic bacterial species numbers decreased following such treatment [8,9]. The combined results of that previous report and of the present study suggest that periodontal disease prevention and bacterial discoloration prevention can be achieved by maintaining a clean intraoral environment through routine checkups and precautions at dental clinics, as well as by daily oral hygiene.



**Figure 5:** The average number of bacterial species per person was calculated for each of the white and discolored groups. Bars indicate the mean  $\pm$  SE values. The statistical difference was determined by a two sided Student's *t* test. \*  $P < 0.01$

### Conclusion

The present study clarified the relationship between tooth

discoloration and the prevalence of periodontopathic bacterial species. The results of the study indicated that maintaining a clean intraoral environment may prevent both periodontal disease and tooth discoloration.

### Acknowledgment

We would like to thank Dr. Masato Mikami (Department of Microbiology, School of Life Dentistry at Niigata, The Nippon Dental University) for providing standard DNA of the periodontopathic bacteria, and for helpful suggestions. This work was supported by JSPS KAKENHI Grant Number 15K2061 and 15K11063.

### References

1. Watts A, Addy M. Tooth discolouration and staining: a review of the literature. *Br Dent J.* 2001; 190(6):309-316.
2. Ronay V, Attin T. Black Stain - A Review. *Oral Health Prev Dent.* 2011; 9(1):37-45.
3. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol.* 1996; 11(4):266-273.
4. Chu SJ, Trushkowsky RD, Paravina RD. Dental color matching instruments and systems. Review of clinical and research aspects. *J Dent.* 2010; 38(2):e2-e16.
5. Brewer JD, Wee A, Seghi R. Advances in color matching. *Dent Clin N Am.* 2004; 48(2):341-358.
6. Geary JL, Kinirons MJ. Use of a common shade guide to test the perception of differences in the shades and value by members of the dental team. *Prim Dent Care.* 1999; 6(3):107-10.
7. Shimomura-Kuroki J, Yamashita K, Shimooka S. *Tannerella forsythia* and the HLA-DQB1 allele are associated with susceptibility to periodontal disease in Japanese adolescents. *Odontology.* 2009; 97(1):32-37. doi: 10.1007/s10266-008-0092-1.
8. Shimomura-Kuroki J, Yamashita-Matsuda K, Miyagawa Y, Shimooka S. Prevalence of Cariogenic and Periodontopathic Bacteria in Japanese Children in the Primary and Mixed Dentitions. *J Clin Pediatr Dent.* 2011; 36(1):31-36.
9. Chapple ILC, Van der Weijden F, Doerfer C, Herrera D, Shapira L, Polak D, et al. Primary prevention of periodontitis: managing gingivitis. *J Clin Periodontol.* 2015; 42(Suppl 16):S71-S76. doi: 10.1111/jcpe.12366.