Imaging Science in Dentistry 2023; 53: 209-16
https://doi.org/10.5624/isd.20220208

Clinical assessment of various imaging systems for dental plaque scoring after the use of 3 different toothpastes

Simge Meseli[1], Sergen Ekenel[2], Bora Korkut[1], Burak Aksu[3], Dilek Tagtekin[1,*], Funda Yanikoglu[4]

[1]Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Türkiye
[2]Department of Orthodontics, Faculty of Dentistry, Marmara University, Istanbul, Türkiye
[3]Department of Clinical Microbiology, School of Medicine, Marmara University, Istanbul, Türkiye
[4]Department of Restorative Dentistry, Faculty of Dentistry, Istanbul Kent University, Istanbul, Türkiye

ABSTRACT

Purpose: This study was conducted to compare dental plaque scores obtained through clinical examinations and various imaging techniques, as well as to assess the effectiveness of herbal and conventional toothpastes for plaque removal.

Materials and Methods: Thirty volunteers were divided into 3 groups. Each group was given a different toothpaste (from 2 herbal toothpastes and a conventional toothpaste) with which to brush their teeth for 21 days. Both initially and after brushing, dental plaque samples were collected, and plaque on the buccal surfaces of anterior teeth was scored using several imaging systems after staining with a disclosing agent. Specifically, digital dental photography, intraoral digital scanning, and FluoreCam imaging were employed to capture intraoral images. The Turesky Modified Quigley-Hein Plaque Index was used for clinical examination and image analysis. Quantitative polymerase chain reaction analyses and correlational assessments between clinical examination and imaging scores were conducted before and after toothpaste use. The Shapiro-Wilk test and Pearson correlations were utilized.

Results: The lowest mean value was observed in the clinical examination without staining, while the highest was obtained using the FluoreCam method. No significant change was found in the level of any microorganism assessed following toothpaste use ($P < 0.05$), with the exception of a decrease in *S. mutans* levels after using conventional toothpaste ($P < 0.05$).

Conclusion: Herbal toothpaste demonstrated plaque-removal effectiveness comparable to that of conventional toothpaste. The use of imaging methods for measuring plaque index has been suggested as a means to educate patients about plaque control and promote ongoing oral care. ***(Imaging Sci Dent 2023; 53: 209-16)***

KEY WORDS: Dental Plaque; Photography, Dental; Imaging, Three-Dimensional; Fluorescence

Introduction

Bacterial dental plaque is a biofilm composed of microorganisms, as well as organic and inorganic components, that adhere to the soft and hard tissues of the oral cavity.[1] The relationship between oral biofilm and gingivitis was introduced by Brown and Löe.[2] Dental plaque is known to cause periodontal diseases, so it must be removed from the mouth at regular intervals. Although mechanical cleaning is the most effective method for removing microbial dental plaque, toothpaste serves as an essential aid in this process. Various chemicals are added to toothpastes to enhance plaque removal and provide antimicrobial activity. The most common chemical used in toothpaste is sodium lauryl sulfate (SLS).[3] Recently, natural products such as ginger, hemp seed oil, and propolis have been incorporated to impart antimicrobial activity to herbal toothpastes.[4] Numerous indices have been developed to evaluate the pres-

*This study was supported by the Marmara University Scientific Research Projects Commission with the project number TDH-2021-10163.
Received November 27, 2022; Revised April 17, 2023; Accepted April 24, 2023
Published online June 23, 2023
*Correspondence to : Prof. Dr. Dilek Tagtekin
Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, Basibuyuk Street 9/3 34854, Basibuyuk, Maltepe. Istanbul, Türkiye
Tel) 90-542-312-4294, E-mail) dtagtekin@marmara.edu.tr

Copyright © 2023 by Korean Academy of Oral and Maxillofacial Radiology
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Imaging Science in Dentistry · pISSN 2233-7822 eISSN 2233-7830

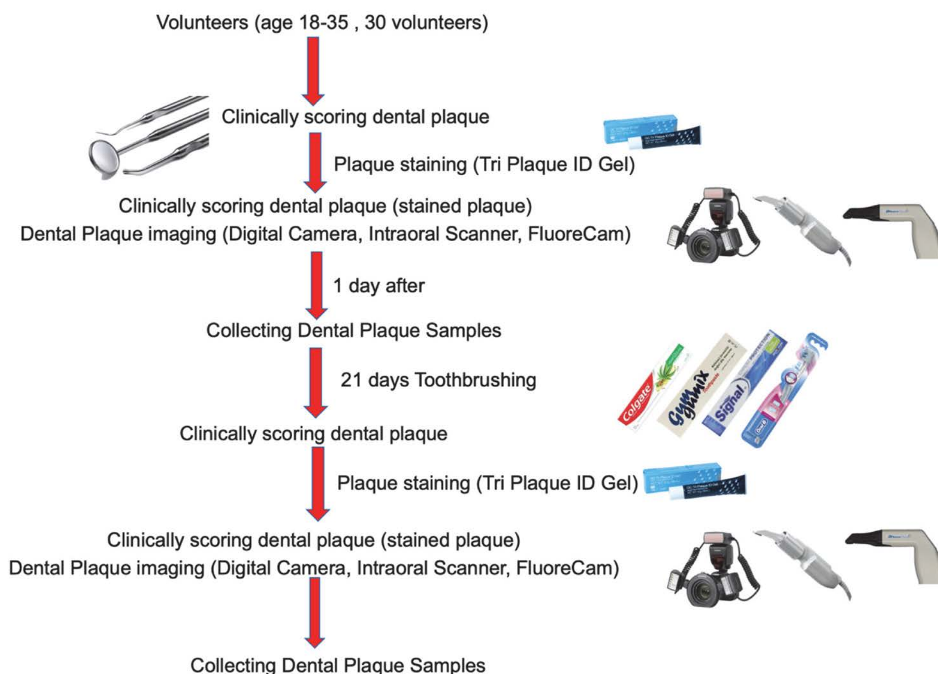

Fig. 1. Study workflow.

ence of microbial dental plaque. According to the Turesky Modified Quigley-Hein Plaque Index, the buccal surfaces of the anterior teeth are divided into 3 parts with an imaginary line, and scores from 0 to 5 are assigned based on the degree of plaque coating on the tooth surface. The plaque score index for an individual is determined by dividing the total score by the number of surfaces examined.[5] Microbial dental plaque is colorless but can be made visible with certain staining agents. One such agent, the GC Tri Plaque ID Gel (GC Corp, Tokyo, Japan), is used in clinics to stain dental plaque, which appears in 3 colors: red, purple, and light blue.[6] Patients should receive oral hygiene education to ensure the proper removal of dental plaque.[7] The use of imaging systems in oral hygiene education enhances its effectiveness. Digital dental photography, intraoral digital scanning, quantitative light-induced fluorescence-digital (QLF-D) imaging (Inspector Research Systems, Amsterdam, The Netherlands), and the FluoreCam System (Therametric, Noblesville, IN, USA) can all be employed for visualizing dental plaque.

Digital dental photography is the most common method for maintaining patient records in dentistry. A fluorescence method, QLF-D, can be utilized to detect initial caries as well as to quantify areas covered by dental plaque. Furthermore, QLF-D involves the use of red fluorescence to identify dental plaque caused by porphyrin produced by oral bacteria, enabling objective detection of even minor changes in plaque. Recent studies have suggested that dental plaque scoring with the QLF-D method correlates with clinical manual scoring methods.[8] One of the fluorescence methods employed in diagnosing microbial dental plaque is FluoreCam. The working system of FluoreCam is the same as that of the QLF-D method. Images of dental plaque, taken after the staining of teeth with a plaque staining agent, are recorded by software in the FluoreCam system. Although FluoreCam is primarily used to diagnose changes in enamel, white spot lesions, and demineralization, it may also serve as an auxiliary method for dental plaque imaging.[9,10] Digital imaging systems, developed for restorative applications, enable computerized 3-dimensional visualization of prepared teeth and restoration design by recording measurements. These systems have led to positive advancements in dentistry. One technique, intraoral digital scanning, allows dentists to view intraoral images of patients in 3 dimensions in just a few minutes.[11-14] Results from a recent study suggest that intraoral digital scanners may be used for dental plaque diagnosis.[15]

Dental plaque can harbor up to 100 different bacterial species at a single site, potentially leading to the development of periodontal disease.[16] Among those bacteria, tooth decay-causing pathogens such as *Streptococcus mutans* (*S. mutans*), *Lactobacillus acidophilus* (*L. acidophilus*), and *Actinomyces viscosus* (*A. viscosus*) are of particular importance.[17]

Determining the bacterial composition of dental plaque samples is challenging due to their complex nature. Various methods are employed for this purpose, including conventional cultivation, quantitative polymerase chain reaction (qPCR), and next-generation sequencing. While traditional diagnosis of oral microorganisms relies on cultivation methods, approximately 50% of oral bacteria cannot be cultured under *in vitro* conditions. In contrast, molecular methods such as qPCR and next-generation sequencing offer substantial advantages in identifying bacterial components.[18,19]

This study was conducted to assess the efficacy of plaque removal by herbal and conventional toothpastes through clinical examination and various imaging techniques. Additionally, the concentrations of 3 distinct pathogenic microorganisms in dental plaque were determined using the qPCR method.

Materials and Methods

This study received approval from the Ethics Committee of Marmara University Faculty of Dentistry (1.10.2020, approval no: 2020-60). It involved 30 adult patients aged 18 to 30 years, recruited from the outpatient population of the Restorative Dentistry Department at Marmara University Faculty of Dentistry. Participants agreed to the terms of the experiment, had a DMFT (D: decayed, M: missing, F: filling, T: teeth) score of 3 or lower, and exhibited relatively even teeth arrangement. Patients with orthodontic brackets, severe tooth crowding that could not be visually verified in photographs, fixed or implant restorations, DMFT scores higher than 3, and serious systemic diseases were excluded from the study.

For standardization, participants were instructed not to consume any food or caloric beverages or engage in oral hygiene practices after dinner. Plaque samples, which formed in the mouth for at least 8 to 12 hours, were visualized between 8:30 and 10:00 AM. Dental plaque samples were collected for microbiological examination after the participants provided informed consent. The workflow of this study is illustrated in Figure 1.

Dental plaque scoring

During the initial session, the Turesky Modified Quigley-Hein Plaque Index was clinically measured using a periodontal probe to detect any plaque accumulation. After assessing the plaque index, teeth were stained with a dental plaque staining agent (Tri Plaque ID Gel; GC Corporation). Following the staining procedure, intraoral photographs were taken from 30 cm away using a professional digital camera (Canon EOS 700D, Canon Inc, Tokyo, Japan; settings: ISO 400, focal length 22, and aperture value 1/125) and a macro lens (Canon EF 100 mm 1 : 2.8 L IS; Canon Inc) to score the stained dental plaque (Fig. 2). After the intraoral photographs were captured, images of the vestibule surfaces of the maxillary and mandibular anterior teeth were recorded using FluoreCam (Fig. 3). All surfaces of the plaque-stained teeth were visualized and documented with an intraoral scanner (iTero Elements 2; Align Technologies, San Jose, CA, USA) (Fig. 4).

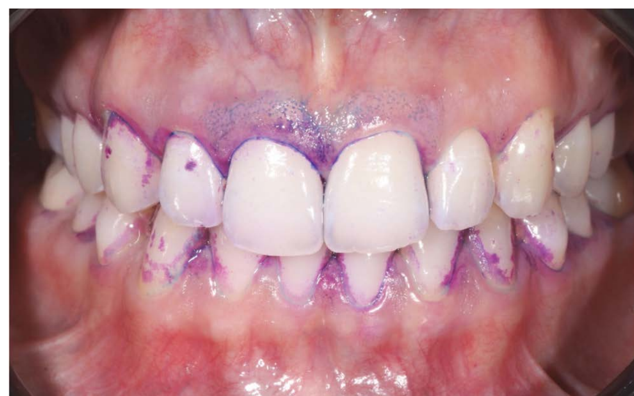

Fig. 2. Intraoral photograph of a participant captured using a professional digital camera (Canon EOS 700D; Canon Inc, Tokyo, Japan) and a macro lens (Canon EF 100 mm 1 : 2.8 L IS; Canon Inc) after the application of a dental plaque staining agent.

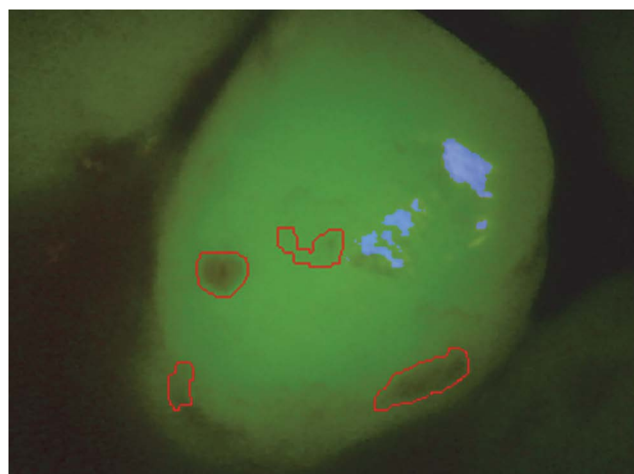

Fig. 3. FluoreCam image captured from tooth #12 of a participant following the application of a dental plaque staining agent.

The day after dental plaque imaging and scoring, plaque samples were obtained from the teeth of participants to investigate alterations in bacterial composition within the dental plaque using qPCR. These samples were collected

using a periodontal curette and subsequently placed in Eppendorf tubes containing distilled water. Throughout the study, the Eppendorf tubes were maintained at a temperature of −80°C.

The 30 participants were randomly assigned to 3 groups. Each group was given 1 of the following toothpastes: an herbal toothpaste containing hemp seed oil (Colgate Hemp Seed Oil; Colgate-Unilever, New York, USA), an herbal toothpaste containing ginger (Gumgumix; Beka Drug, Istanbul, Turkey), or a conventional toothpaste (Signal Expert Protection; Unilever, Sofia, Bulgaria) (Table 1). Participants were instructed to brush their teeth twice daily for 21 days using a standard toothbrush provided to them and the modified toothpaste technique. In this method, toothpaste is applied evenly to the teeth using the fingers before brushing.[20] After 21 days, the dental plaque scoring and imaging procedures were repeated to obtain a second set of measurements. Dental plaque samples were again collected and placed in Eppendorf tubes. Bacterial changes in the samples taken at the initial session and after 21 days of toothbrushing were examined using qPCR at Marmara University Faculty of Medicine, Department of Clinical Microbiology. The levels of *S. mutans*, *L. acidophilus*, and *A. viscosus* in the plaque samples were quantified.

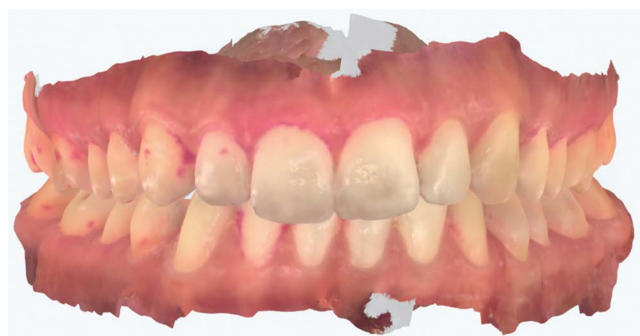

Fig. 4. Intraoral scanning image of a participant captured by an intraoral scanner (iTero Elements 2; Align Technologies, San Jose, CA, USA) following the application of a dental plaque staining agent.

Quantitative real-time PCR analysis

The levels of cariogenic pathogens, including *S. mutans*, *L. acidophilus*, and *A. viscosus*, in dental plaque samples were investigated using qPCR. Each plaque sample was suspended in 0.1 mL of sterile DNase-/RNase-free water. Bacterial genomic DNA was isolated from these suspensions using the boiling method and served as a template for PCR amplification. The PCR reaction was prepared in a total volume of 25 μL, consisting of 12.5 μL GM Sybr qPCR Kit with Sybr Green dye (GeneMark; GMbiolab, Taichung, Taiwan), 1 μM of each primer described above, and 1 μL of the target DNA. Amplification reactions were carried out in a real-time thermal cycler (RotorgeneQ; Qiagen, Hilden, Germany). PCR conditions included initial denaturation for 5 minutes at 95°C, followed by 30 cycles at 95°C for 45 seconds and 60°C for 45 seconds for *S. mutans* and *A. viscosus*; 30 cycles at 95°C for 45 seconds and 56°C for 45 seconds were used for *L. acidophilus*. A 100-fold dilution series was prepared for genomic DNA from the standard strain of each bacterial pathogen, ranging from 1×10^6 to 1×10^2 CFU/mL, and used as quantitation standards in the real-time PCR experiments.[21]

Statistical analyses

Statistical analyses were conducted using SPSS version 23 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was employed to assess conformity to the normal distribution. Pearson correlations were computed to compare changes by group. Linear models were employed to compare dental plaque values by group, time, and method. The Tukey honestly significant difference test was used for multiple comparisons. In the qPCR analysis, the Kru-

Table 1. Toothpaste ingredients used in the study

Toothpastes	Ingredients
Signal expert protection (Unilever, Sofia, Bulgaria)	Sodium monofluorophosphate, silica, potassium citrate, zinc citrate, hydroxyapatite, PEG-32, sodium lauryl sulfate, trisodium phosphate, cellulose gum, sodium hydroxide, sodium saccharin, Cl 74160, Cl 77891
Colgate hemp seed oil (Colgate-Palmolive, New York, USA)	Sodium fluoride, sorbitol, water, hydrated silica, PEG-12, sodium lauryl sulfate, flavor, cellulose gum, sodium saccharin, tetrasodium pyrophosphate, cocamidopropyl betaine, hemp seed oil
Gumgumix (Beka Drug, Istanbul, Türkiye)	Calcium carbonate, glycerin, water, honey, licorice root, dicalcium phosphate, ginger extract, xanthan gum, sodium carboxymethyl, potassium sorbate, menthol, sodium benzoate

Table 2. Descriptive statistics dental plaque scores according to plaque scoring methods

Group	Time	Clinical (not staining)	Clinical (after staining)	Intraoral scanner	Digital camera	FluoreCam	Total
GumGumix	Initial	0.48±0.67	0.92±0.58	0.91±0.53	0.92±0.58	1.24±0.42	0.89±0.59
	After brushing	0.66±0.70	1.07±0.56	0.89±0.55	1.07±0.56	1.56±0.74	1.05±0.67
	Total	0.57±0.67	1.00±0.56	0.90±0.52	1.00±0,56	1.4±0.61	0.97±0.63[b]
Signal	Initial	0.37±0.25	0.92±0.26	0.85±0.31	0.92±0.26	1.0±0.50	0.91±0.48
	After brushing	0.40±0.19	0.84±0.29	0.72±0.36	0.84±0.29	1.44±0.49	0.85±0.47
	Total	0.39±0.22	0.88±0.27	0.79±0.34	0.88±0.27	1.47±0.48	0.88±0.47[b]
Colgate	Initial	0.69±0.43	1.22±0.67	1.16±0.31	1.22±0.67	1.76±0.52	1.21±0.62
	After brushing	0.70±0.43	1.25±0.66	1.12±0.25	1.25±0.66	1.77±0.52	1.22±0.61
	Total	0.69±0.42	0.69±0.42	1.14±0.28	1.23±0.65	1.76±0.51	1.21±0.61[a]
Total	Initial	0.52±0.48	1.02±0.53	0.97±0.40	1.02±0.53	1.50±0.51	1.01±0.58
	After brushing	0.58±0.49	1.05±0.54	0.91±0.43	1.05±0.54	1.59±0.59	1.04±0.61
	Total	0.55±0.48[c]	1.04±0.53[b]	0.94±0.41[b]	1.04±0.53[b]	1.54±0.55[a]	1.02±0.59

[a,b,c]: No significant difference between groups and methods with the same letter

Table 3. Bacterial burden for *S. mutans* quantified by qPCR in 21 days brushing (unit: CFU/μL)

		Initial	After brushing	p**
Gumgumix	Mean	$7.3\times10^4\pm8.9\times10^4$	$6.3\times10^4\pm6.4\times10^4$	0.646
	Median (Max.-Min.)	5.2×10^4 (2.1×10^3-2.9×10^5)	5.2×10^4 (2.3×10^3-2.1×10^5)	
Signal	Mean	$4.8\times10^4\pm8.2\times10^4$	$2.2\times10^4\pm3.4\times10^4$	<0.05
	Median (Max.-Min.)	1.1×10^4 (1.8×10^3-2.6×10^5)	2.4×10^3 (8.8×10^2-1×10^5)	
Colgate	Mean	$4.3\times10^4\pm4.2\times10^4$	$2.9\times10^4\pm2.9\times10^4$	0.203
	Median (Max-Min.)	3.3×10^4 (1.6×10^3-1.2×10^5)	1.7×10^4 (6.5×10^2-7.8×10^4)	
	p*	0.538	0.079	

*Kruskal Wallis H Test, **Wilcoxon Test, qPCR: quantitative polymerase chain reaction, CFU: colony-forming unit

skal-Wallis *H* test was applied to compare non-normally distributed data. The Wilcoxon test was employed for data that did not fit a normal distribution when comparing 2 dependent groups. The analyzed results were presented as mean ± standard deviation for quantitative data and as a percentage of frequency for categorical data ($P<0.05$).

Results

Dental plaque imaging results

The plaque score values for anterior teeth were compared with regard to toothpaste group, time, and imaging method. A difference in plaque values was noted among the groups, independent of time and method ($P<0.05$). The mean plaque values were 0.97 in the Gumgumix group, 0.88 in the Signal group, and 1.21 in the Colgate group; in other words, lower mean values were found in the Gumgumix and Signal groups than in the Colgate group. Independent of the group and method, no significant difference was found in plaque values over time. However, a difference in plaque values was noted across methods, independent of group and time. The mean values were 0.55 for the clinical plaque-unstained group, 1.04 for the clinical plaque-stained group, 0.94 for the scanning plaque-stained group, 1.04 for the digital camera group, and 1.54 for the FluoreCam group. In other words, a lower mean value was associated with the clinical plaque-unstained method and a higher mean value with the FluoreCam method, relative to the other imaging techniques. No significant differences in plaque values were

Table 4. Bacterial burden for *L. acidophilus* quantified by qPCR in 21 days brushing (unit: CFU/μL)

		Initial	After brushing	p**
Gumgumix	Mean Median (Max.-Min.)	$6.8\times10^5\pm1\times10^6$ 3.5×10^5 $(1.6\times10^4$-$3.6\times10^6)^a$	$6.2\times10^5\pm8.9\times10^5$ 3.1×10^5 $(9.8\times10^4$-$2\times10^6)^a$	0.721
Signal	Mean Median (Maks.-Min.)	$2.2\times10^6\pm2.1\times10^6$ 1.7×10^6 $(1.2\times10^5$-$5.3\times10^6)^{ab}$	$2\times10^6\pm1.8\times10^6$ 1.6×10^6 $(1.2\times10^5$-$7\times10^6)^{ab}$	0.799
Colgate	Mean Median (Maks.-Min.)	$7.9\times10^6\pm9.6\times10^6$ 4.9×10^6 $(6.1\times10^4$-$2.9\times10^7)^b$	$6.3\times10^6\pm7.6\times10^6$ 3.8×10^6 $(5.8\times10^4$-$2.4\times10^7)^b$	0.059
	p*	0.011	0.010	

*Kruskal Wallis H Test, **Wilcoxon Test, a,b: No difference between groups with the same letter, qPCR: quantitative polymerase chain reaction, CFU: colony-forming unit

Table 5. Bacterial burden for *A. viscosus* quantified by qPCR in 21 days brushing (unit: CFU/μL)

		Initial	After brushing	p**
Gumgumix	Mean Median (Max-Min.)	$1.4\times10^5\pm5.5\times10^4$ 1.4×10^5 $(5.5\times10^4$-$2.3\times10^5)$	$1.1\times10^5\pm5.3\times10^4$ 1×10^5 $(4.9\times10^4$-$2\times10^5)$	0.139
Signal	Mean Median (Max.-Min.)	$9.9\times10^4\pm3.4\times10^4$ 1×10^5 $(4.9\times10^4$-$1.6\times10^5)$	$9.2\times10^4\pm5.8\times10^4$ 6.4×10^4 $(4\times10^4$-$1.9\times10^5)$	0.646
Colgate	Mean Median (Maks-Min.)	$1.2\times10^5\pm6.7\times10^4$ 9.9×10^4 $(5.9\times10^4$-$2.5\times10^5)$	$9.6\times10^4\pm2.6\times10^4$ 9.8×10^4 $(5.9\times10^4$-$1.4\times10^5)$	0.508
	p*	0.083	0.750	

*: Kruskal Wallis H Test, **: Wilcoxon test, qPCR: quantitative polymerase chain reaction, CFU: colony-forming unit

observed according to the interactions between group and time, group and method, or time and method. In terms of plaque scoring methods, no significant difference was found between plaque score values at baseline and after 21 days of brushing in all toothpaste groups (Table 2).

qPCR results

In comparing the levels of *S. mutans*, no significant difference was observed between the qPCR values obtained in the initial session across groups ($P>0.05$). However, a significant difference was found between the initial and post-brushing values obtained in the Signal group ($P<0.05$) (Table 3). In comparing the levels of *L. acidophilus*, a significant difference was observed between the qPCR values obtained in the initial session across the groups ($P<0.05$). However, when comparing the levels of *A. viscosus*, no difference was found between the qPCR values obtained in the initial session across groups ($P>0.05$). Although a decrease was observed in the quantity of *L. acidophilus* and *A. viscosus* after brushing, no statistically significant difference was detected for any toothpaste groups (Tables 4 and 5).

Discussion

Most people struggle to maintain effective oral hygiene, which predisposes them to oral infections such as periodontal disease and dental caries. Since these diseases are caused by dental plaque bacteria, it is crucial to control their growth and colonization. Numerous chemical agents have been added to toothpastes to prevent the growth and colonization of microorganisms in the oral cavity. SLS is the most widely used antiplaque agent found in toothpastes.[22] In this study, the antibacterial effects of a conventional toothpaste containing SLS and fluoride (Signal Expert Protection) were compared to those of 2 herbal toothpastes containing hemp seed oil and/or ginger (Colgate Hemp Seed Oil; Gumgumix) on the teeth of participants. Hemp seed oil and ginger are herbal products added to toothpastes for their antibacterial properties.

People who lack proper oral hygiene habits often struggle with consistently brushing their teeth. To ensure a standardized oral hygiene status, this study included participants who demonstrated good oral hygiene and had DMFT scores of 3 or lower. The index serves as a measurement method to determine the amount or severity of a disease or its agent in an individual or society. These indexes are utilized to track changes in a patient's periodontal status, compare incidence rates across populations, and assess the effectiveness of various therapeutic methods.[2,7,23,24] In this study, the Turesky Modified Quigley-Hein Plaque Index was used to measure the initial plaque scores of participants, as it can be easily recorded without special equipment, provides detailed information on plaque presence on tooth surfaces, and allows for quick and clear scoring.[6]

Nandlal et al.[25] examined the impact of an herbal toothpaste and a conventional fluoride toothpaste on dental plaque, assessing dental plaque scores using the Turesky Modified Quigley-Hein Plaque Index before and after toothpaste application. That study found no significant difference in the change of dental plaque scores between the 2 toothpastes. This finding aligns with the present research, which also indicated no significant difference between the groups using conventional and herbal toothpaste in terms of dental plaque index measurements taken before and after toothpaste usage.

In the present study, a plaque staining agent (Tri Plaque ID Gel) was utilized after the initial manual measurement to make dental plaque visible. While the literature contains numerous studies comparing plaque indices, fewer studies have compared dental plaque imaging methods. Research investigating dental plaque imaging using digital intraoral scanning and the FluoreCam method has not yet been published. The present study is the first to compare clinical scoring and various imaging systems in the diagnosis of dental plaque.

Lee et al.[8] examined the relationship between QLF-D scores and patient hygiene performance plaque indices, which were measured after dental plaque staining. They discovered a correlation between QLF-D scores and clinical scoring, a finding that aligns with the present study. In the present research, the FluoreCam method, which operates similarly to QLF, was compared with the dental plaque index obtained after application of a plaque staining agent. The results indicated that the plaque scores acquired using the FluoreCam method were higher than those recorded after plaque staining in a clinical setting. This discrepancy may be due to the FluoreCam system's capacity to magnify individual teeth, thereby providing a more detailed view of dental plaque and producing higher plaque scores.

In a study comparing the correlation between QLF-D imaging and PCR analysis, supragingival plaque samples from patients were analyzed using PCR after dental plaque deposits were visualized with the red fluorescence of QLF-D. The researchers observed a higher rate of periodontopathogenic bacteria in PCR results for individuals with high red fluorescent dental plaque using QLF-D.[26] The present study employed the FluoreCam method, which operates on the same principle as QLF, as an imaging technique. Based on the images captured with FluoreCam, plaque scores decreased the most in the group using conventional toothpaste (Signal Expert Protection). Similar results were obtained for dental plaque scores generated by both clinical visual and imaging methods, in terms of scores before and after toothpaste usage. Consequently, in the qPCR examination of dental plaque samples, the levels of all microorganisms assessed in the study were deemed similar after toothpaste usage relative to before, with the exception of a decrease in *S. mutans* levels following the use of conventional toothpaste (Signal Expert Protection).

Diagnosing dental plaque quickly is challenging, and the process is prone to error. However, new imaging systems enable image enlargement, which allows for more accurate detection of dental plaque. Visualizing dental plaque is not only helpful for clinicians but also motivating for patients. The combination of intraoral digital scanning images, fluorescence methods, and digital dental photographs significantly enhances the diagnosis and imaging of dental plaque. Intraoral digital scanning offers a distinct advantage in diagnosing dental plaque due to its 3-dimensional imaging capabilities. Furthermore, the fluorescence method provides a quantitative evaluation. Separately, the present study indicates that herbal toothpastes demonstrate comparable plaque-removal effectiveness to conventional toothpaste. The similar results for microorganism levels after toothpaste use may be attributed to the study's inclusion of participants with low DMFT and low dental plaque scores. For future studies, it would be advantageous to include participants with high DMFT and dental plaque scores, as well as to expand the variety of microorganisms evaluated in dental plaque.

Conflicts of Interest: None

References

1. Larsen T, Fiehn NE. Dental biofilm infections - an update. APMIS 2017; 125: 376-84.

2. Brown LJ, Löe H. Prevalence, extent, severity and progression of periodontal disease. Periodontol 2000 1993; 2: 57-71.
3. Lippert F. An introduction to toothpaste - its purpose, history and ingredients. Monogr Oral Sci 2013; 23: 1-14.
4. Kanth MR, Prakash AR, Sreenath G, Reddy VS, Huldah S. Efficacy of specific plant products on microorganisms causing dental caries. J Clin Diagn Res 2016; 10: ZM01-3.
5. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol 1970; 41: 41-3.
6. Brostek AM, Walsh LJ. Minimal intervention dentistry in general practice. Oral Health Dent Manag 2014; 13: 285-94.
7. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Newman and Carranza's clinical periodontology. 13th ed. Philadelphia, PA: Elsevier; 2019.
8. Lee JB, Choi DH, Mah YJ, Pang EK. Validity assessment of quantitative light-induced fluorescence-digital (QLF-D) for the dental plaque scoring system: a cross-sectional study. BMC Oral Health 2018; 18: 187.
9. Kiriştioğlu Z, Yanikoglu F, Alkan E, Tağtekin D. The effect of dental paste with herbal content on remineralization and the imaging with fluorescent technique in teeth with white spot lesion. Clin Exp Health Sci 2021; 11: 348-53.
10. Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. Prevention of demineralization around orthodontic brackets in vitro. Am J Orthod Dentofacial Orthop 2007; 131: 705. e1-9.
11. Logozzo S, Franceschini G, Kilpelä A, Caponi M, Governi L, Blois L. A comparative analysis of intraoral 3D digital scanners for restorative dentistry. Internet J Med Technol [Internet] 2011 [cited Nov 20]; 5 (1): Available from: https://ispub.com/IJMT/5/1/10082.
12. Imburgia M, Logozzo S, Hauschild U, Veronesi G, Mangano C, Mangano FG. Accuracy of four intraoral scanners in oral implantology: a comparative in vitro study. BMC Oral Health 2017; 17: 92.
13. Hurt AJ. Digital technology in the orthodontic laboratory. Am J Orthod Dentofacial Orthop 2012; 141: 245-7.
14. Commer P, Bourauel C, Maier K, Jäger A. Construction and testing of a computer-based intraoral laser scanner for determining tooth positions. Med Eng Phys 2000; 22: 625-35.
15. Doi K, Yoshiga C, Kobatake R, Kawagoe M, Wakamatsu K, Tsuga K. Use of an intraoral scanner to evaluate oral health. J Oral Sci 2021; 63: 292-4.
16. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005; 43: 5721-32.
17. Pereira CA, Romeiro RL, Costa AC, Machado AK, Junqueira JC, Jorge AO. Susceptibility of *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus mutans* biofilms to photodynamic inactivation: an in vitro study. Lasers Med Sci 2011; 26: 341-8.
18. Li Y, Zhang Q, Zhang F, Liu R, Liu H, Chen F. Analysis of the microbiota of black stain in the primary dentition. PloS One 2015; 10: e0137030.
19. Çelik ZC, Çakiris A, Yanıkoğlu F, Abacı N, Ekmekçi SS, Ilgın C, et al. Metagenomic analysis of black-stained plaques in permanent dentition. Arch Oral Biol 2021; 128: 105171.
20. Sjögren K. Toothpaste technique. Studies on fluoride delivery and caries prevention. Swed Dent J Suppl 1995; 110: 1-44.
21. Lochman J, Zapletalova M, Poskerova H, Izakovicova Holla L, Borilova Linhartova P. Rapid multiplex real-time pcr method for the detection and quantification of selected cariogenic and periodontal bacteria. Diagnostics (Basel) 2019; 10: 8.
22. Herlofson BB, Barkvoll P. Oral mucosal desquamation caused by two toothpaste detergents in an experimental model. Eur J Oral Sci 1996; 104: 21-6.
23. Ramfjord S, Emslie R, Greene JC, Held A, Waerhaug J. Epidemiological studies of periodontal diseases. Am J Public Health Nations Health 1968; 58: 1713-22.
24. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962; 65: 26-9.
25. Nandlal B, Sreenivasan PK, Shashikumar P, Devishree G, Bettahalli Shivamallu A. A randomized clinical study to examine the oral hygiene efficacy of a novel herbal toothpaste with zinc over a 6-month period. Int J Dent Hyg 2021; 19: 440-9.
26. Lee ES, de Josselin de Jong E, Kim BI. Detection of dental plaque and its potential pathogenicity using quantitative light-induced fluorescence. J Biophotonics 2019; 12: e201800414.