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Effects of chlorhexidine (gel) application on bacterial levels and orthodontic brackets during orthodontic treatment

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Abstract: The objectives of this study were to evaluate the effects of applying 0.50% chlorhexidine (CHX) gel using the dental drug delivery system (3DS) on salivary *Streptococcus mutans* (*S. mutans*) and on the surface topography of metal and ceramic orthodontic brackets. The study involved 20 orthodontic patients with high levels of salivary *S. mutans*. The patients were treated with professional mechanical tooth cleaning followed by application of 0.50% CHX using individual trays (3DS). Salivary *S. mutans* levels were repeatedly measured 1, 2, 4, and 8 weeks post-treatment. *In vitro* study utilized forty ceramic and metallic brackets that were immersed in 0.50% CHX gel for 10 min, whereas another untreated forty brackets served as controls. The frictional resistances of stainless steel wires to the brackets before and after CHX treatment were recorded using a universal testing machine. Scanning electron microscopy was used to compare changes in the surface topography of brackets. Statistical analyses were used to determine the effect of CHX on bacterial count and to evaluate the effect of CHX on frictional resistance. According to the results of this study, *S. mutans* levels were reduced significantly ($P < 0.05$). There were no significant changes in the frictional resistance and surface topography of brackets before or after application of CHX. (J Oral Sci 58, 35-42, 2016)

Keywords: chlorhexidine; *Streptococcus mutans*; frictional resistance; orthodontic brackets; dental drug delivery system.

Introduction

Orthodontic treatment is considered to improve the self-image of patients by providing more esthetically pleasing and attractive smiles. Despite the post-therapeutic esthetic advantages of orthodontics, the treatment is associated with an increase in caries incidence because orthodontic brackets form sites of retention for dental biofilm and also interfere with proper tooth cleaning (1).

Dental biofilm formation is a result of an increase in the number of microorganism that is directly related to dental caries, particularly *Streptococcus mutans* (*S. mutans*) (2-7). Dental biofilm formation on tooth surfaces is 2-3 times higher in orthodontic patients than high plaque-forming adults with no orthodontic treatment (1). Because dental biofilm is the cause of various oral diseases, its removal and control are critical for oral health maintenance (1,8). Previous research has shown that regular tooth brushing and use of dental floss alone do not decrease the levels of salivary *S. mutans* in patients

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treated with fixed orthodontic appliances (9).

Different protocols using antimicrobial agents, particularly chlorhexidine (CHX), have been introduced to help control biofilm formation on teeth and brackets in patients treated with fixed orthodontic appliances (4,10,11). CHX has the ability to inhibit dental biofilm formation and to decrease the level of salivary *S. mutans*, which is considered the main causative bacteria responsible for the development of dental caries (12-14).

Some studies have reported that application of CHX was not successful in decreasing the cariogenic activity of *S. mutans* because of the ability of these bacteria to secrete extracellular glucans through the action of the bacterial glucotransferase enzyme (6). Glucans are insoluble polysaccharides that shield *S. mutans* from the bactericidal effects of CHX and limit the CHX concentrations reaching the bacteria (14). It has been suggested that clinicians should prolong the exposure of dental biofilms to CHX and should increase CHX concentration so that an effective bactericidal concentration against *S. mutans* can be reached (14). However, these techniques of application of CHX have been associated with the bacterial levels being restored to the high baseline levels within 2-12 weeks. This was because of the development of bacterial resistance to CHX (15) or because of the development of various adverse effects on the oral mucosa and imbalance in the oral flora due to application of high concentrations of CHX for long time periods (16).

In an attempt to overcome the aforementioned difficulties in clinical application of CHX for decreasing the levels of salivary *S. mutans*, the dental drug delivery system (3DS) for application of CHX was introduced (17,18). This method relies on thorough professional mechanical tooth cleaning (PMTC) to ensure maximum removal of insoluble glucan layers, followed by application of CHX gel via 3DS to the tooth structure, minimizing any adverse effects on the oral flora.

This technique proved effective in decreasing the levels of oral salivary streptococci and decreased their cariogenic activity (17,18). However, no investigation was performed to examine the efficacy of 3DS at decreasing the levels of salivary streptococci in orthodontic patients, which are classified as high risk for the development of dental caries. In addition, the safety of using CHX on various fixed orthodontic appliances was not reported. It is of prime importance to investigate all the possible adverse factors, which may increase friction (19,20) between orthodontic wires and brackets because it may jeopardize the final outcome of orthodontic treatment planning (19,21-26).

This study aimed to examine the feasibility of applying CHX gel via 3DS to high-risk orthodontic patients and to determine its effectiveness in decreasing the levels of salivary *S. mutans*. Moreover, this study investigated the effect of CHX gel on the surface topography of ceramic and metallic brackets.

Materials and Methods

Patient selection criteria for the *in vivo* experiment

Patient recruitment was performed based on the protocols approved by the Ethics Committee for Research of the Faculty of Dentistry, King Abdulaziz University (Jeddah, Saudi Arabia), registered under the authorization identifier NCT02001311 at clinicaltrial.gov. All procedures involving human participants were performed after obtaining signed informed consents in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments. Twenty volunteers were randomly selected from the records of the orthodontic clinics of the faculty based on the patient inclusion criteria.

Inclusion/exclusion criteria were as follows: 1) patients age range; 20-30 years, 2) patients undergoing fixed orthodontic treatment at the end of the leveling and alignment stage, 3) initial bacterial level indicating high caries risk, 4) all patients were given oral hygiene instruction and dietary advice at the first visit, 5) no patients had taken antibiotics for at least 6 weeks before saliva sampling or during the experimental period, 6) all patients used a fluoride-containing dentifrice twice a day, 7) diet analyses were done to rule out any patients suffering from improper dietary habits, 8) patients with abnormally low salivary flow were excluded from this study.

Dental drug delivery tray fabrication

At screening, alginate impressions were made for all the patients, and maxillary and mandibular casts were prepared. Each tooth on the cast was blocked with paraffin wax to obtain space for drug delivery. A polypropylene sheet (Easy Vac-Gasket, 3A MEDES, Gyeonggi-do, Republic of Korea) was vacuum-adapted to each cast using a vacuum-forming machine (Henry Schein, Henry Schein Inc., NY, USA). From the vacuum adapted sheet, the individual trays, referred to as drug retainers, were made to fit on the tooth surfaces and cover the complete arch of the dentition and were trimmed to be approximately 1 mm above the gingival margin.

Clinical procedures

At the first visit, the salivary concentrations of *S. mutans* were evaluated using a commercially available bacterial evaluation kit (CRT Bacteria, Ivoclar Vivadent, AG

Table 1 Description of the grading system used for bacterial levels

Grade	Caries risk	CFU of *S. mutans* / mL of saliva
1.0	Low	= $<10^4$
2.0	Low	= 10^4-10^5
3.0	High	= 10^5-10^6
4.0	High	>10^6

Table 2 Distribution of stainless-steel and ceramic brackets into chlorhexidine and control groups

Group	Number (*n*)	Bracket type	CHX application
C-Control	20	Ceramic	−
C-CHX	20	Ceramic	+
S-Control	20	Stainless-steel	−
S-CHX	20	Stainless-steel	+

Schaan, Liechtenstein); the bacterial levels were documented according to the manufacturer's instructions, and baseline data were obtained.

At the subsequent visit, PMTC was performed to remove the biofilm on the teeth and bracket surfaces. An ultrasonic scaler (Supuason Satelec Inc., Bordeaux, France) was used to remove supragingival calculus. Dental biofilm was removed by brushing and flossing. Disclosing agents were used frequently to determine the amount of dental biofilm remaining on the surfaces, and the procedure was continued until the teeth were no longer colored by the disclosing agents. Following the complete removal of dental biofilm, all teeth were polished using pumice and a rotary cup or brush.

All patients were then treated with CHX gel (CURASEPT 0.50% Gel, Curaprox, Kriens, Switzerland), which was applied to the tooth surfaces using the removable drug retainer for 5 min. The retainers were then removed, and the patients were asked not to eat or drink for 2 h. The patients were given new toothbrushes and were advised to brush their teeth twice a day using commercially available 0.3% sodium fluoride tooth paste (Crest, Procter & Gamble, Surrey, UK). At 1 week following the first treatment, CHX gel was applied again at the clinic for the same time period.

Bacterial level assessment

The bacterial levels were measured 1, 2, 4, and 8 weeks after completion of treatment and were compared with the baseline data. According to the manufacturer's instructions, the patients chewed on a paraffin pellet to stimulate salivation, and their saliva was collected. A $NaHCO_3$ tablet was placed in the test vial; the tablet released CO_2 when it came in contact with the liquid. This created favorable conditions for bacterial growth. The agar carrier was immediately placed in the test vial, which was tightly sealed. Incubation for 2 days (CRT-incubator from Ivoclar Vivadent) at 37°C was sufficient to allow the bacterial colonies to grow. *S. mutans* colonies were identified as small, blue hemispheres with a diameter of <1 mm on the blue agar. Comparison with the corresponding images in the model chart permitted the assessment of the initial risk of caries as well as before and after application of CHX using 3DS. According to the manufacturer, scores of 1.0 and 2.0 were considered low caries risk, whereas scores of 3.0 and 4.0 were indicative of high caries risk (Table 1).

Frictional resistance examination

In this study, 40 pre-adjusted upper canine ceramic brackets (Clarity ADVANCED, 3M Unitek) and 40 pre-adjusted upper canine metal brackets (Gemini series, 3M Unitek) with the same prescription (0.022 in slot, MBT) were used. A total of 80 stainless steel wires (Permachrome Archwire, 3M Unitek; 0.019 × 0.025 inch) were used.

The wires and brackets were equally divided into four groups. S-CHX (stainless-steel brackets) and C-CHX (ceramic brackets) represented the groups in which the brackets were immersed in 0.50 wt% CHX gel for 10 min. The following two groups served as controls and the brackets in these groups were not exposed to CHX: S-Control (stainless-steel brackets) and C-Control (ceramic brackets) (Table 2).

Stainless steel alloy wire (0.019 × 0.025 inch; Permachrome Archwire, 3M Unitek) was used for laboratory friction test. A total of 80 bracket-wire samples were investigated. Each bracket was tested once, and each wire specimen was drawn through one bracket only to eliminate the influence of wear. Prior to evaluation, each wire and bracket were cleaned with 95% ethanol and dried with compressed air. All the friction tests were conducted in a dry state, in prevailing air, to achieve results in non-contaminated conditions as reported in previous studies (27,28).

All brackets were tested for static and dynamic friction at zero angulations using a Universal Testing Machine (ElectroPlus E1000, Instron, Norwood, MA, USA). A 10-cm straight wire was tied to the bracket by elastic ligation (3M Unitek); the upper end of the wire was connected to the tension-loading cell of the machine, and the lower end remained free (28). The bracket itself was bonded to an ivory tooth that was held by a custom attachment inserted into the adjustable base of the machine. The tested wire was connected to a load cell with a maximum load of 500 kN and was pulled through

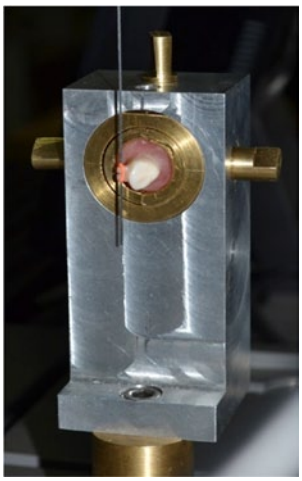

Fig. 1 Bracket and arch-wire in vertical placement on special attachment for measuring frictional resistance.

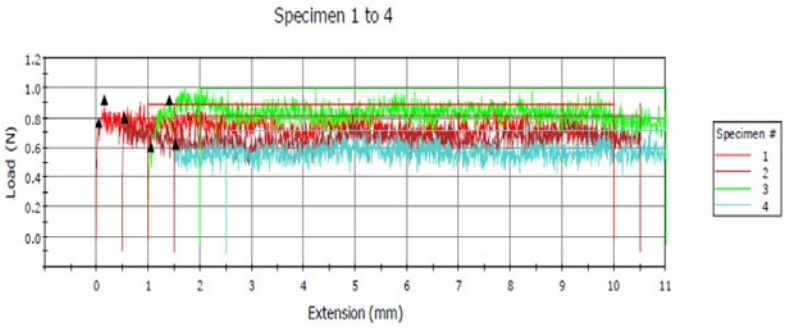

Fig. 2 Load-extension friction data of the four different specimens.

for 10 mm with a crosshead speed of 5 mm/min. Each test was conducted for 2 min (Fig. 1) (27).

The static frictional force was recorded by measuring the maximum force at the initial extension, and the dynamic frictional force was recorded by calculating the average of frictional force on reaching the static friction peak. The load (N) and deflection (mm) were recorded for each specimen using a computer software program (Bluehill software for Instron Mechanical Testing Instruments, Norwood, MA, USA). The load cell registered the forces required to move the wire along the bracket and transmitted them to a computer hard disk. The data were recorded on an XY recorder (Fig. 2); the X-axis recorded the wire movement in millimeters, whereas the Y-axis recorded the frictional force between the bracket and the arch wire in Newtons. Static frictional force was calculated at the initial peak of movement, whereas dynamic frictional force was calculated by averaging five recordings 10 s apart on the Y-axis after the static friction peak (27).

Scanning electron microscope (SEM) evaluation

From each group, five respective brackets were randomly selected and examined using a SEM (ZEISS, Oberkochen, Germany) to evaluate the surface topography of the brackets before and after exposure to CHX and friction tests. The specimens were gold-coated and then examined by SEM at ×2,000 magnification.

Statistical analysis

The percentages of patients exhibiting every grade of *S. mutans* were calculated for each time period. The Friedman test was used to evaluate the effect of using CHX on decreasing the levels of salivary *S. mutans*. To compare the effect of CHX at each follow up post-baseline, the Wilcoxon signed-rank test was used, and a Bonferroni correction was applied. The level of significance was set at $P < 0.05$ for the Friedman test and $P < 0.013$ for the Wilcoxon test.

Descriptive statistics, including the means and standard deviations, were calculated for static and dynamic frictional force values for each bracket type. For static and dynamic frictional force, two-way analysis of variance (ANOVA) was used to evaluate the effect of CHX and type of bracket and their interaction. The level of significance for all the tests was set at $P < 0.05$. All the statistical analyses were performed using STATA version 13 (StataCorp, College Station, TX, USA).

Results

In vivo bacterial level assessment

No adverse effects were noted in the *in vivo* study. There was no increase in staining of teeth or other oral surfaces. There was no increase in calculus formation and no alterations in taste perception during the experimental period. Furthermore, there were no reports of debonding brackets.

The distribution of the *S. mutans* grades at baseline and during follow up are listed in Table 3 and Fig. 3. There was a statistically significant difference in levels of salivary *S. mutans* between the different time periods ($P < 0.001$). Post-hoc multiple comparison tests revealed that the decrease in *S. mutans* grades at each follow-up time period were significantly lower than that at baseline ($P < 0.001$). The most marked reduction was seen in week 1 with 65% patients scoring grade 2.0, which indicated low caries risk.

Table 3 The frequency (percentages) and means (standard deviations) of *Streptococcus mutans* grades at baseline and following application of chlorhexidine (1, 2, 4, and 8 weeks post-treatment)

S. mutans grade*	Baseline *n* (%)	Follow up time (in weeks) after treatment *n* (%)			
		1**	2**	4**	8**
1	0	3 (15)	2 (10)	5 (25)	2 (10)
2	0	13 (65)	12 (60)	9 (45)	12 (60)
3	6 (30)	3 (15)	5 (25)	5 (25)	3 (15)
4	14 (70)	1 (5)	1 (5)	1 (5)	3 (15)
Mean (±SD)	3.7 (±0.5)	2.1 (±0.7)	2.3 (±0.7)	2.1 (±0.9)	2.4 (±0.9)

*Friedman test of significance of CHX treatment on *S. mutans* grade: $P < 0.05$

**Wilcoxon singed-rank test of significance of CHX treatment on *S. mutans* grades comparing follow up time (after treatment) to baseline: $P < 0.013$

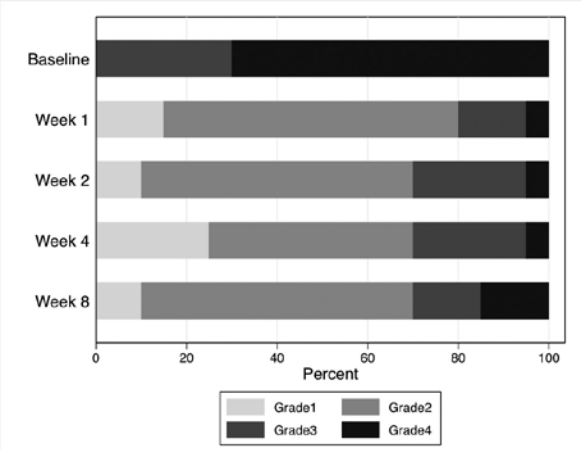

Fig. 3 Distribution of *Streptococcus mutans* grades before (baseline) and after application of chlorhexidine (1, 2, 4, and 8 weeks post-treatment).

Table 4 Comparison of the means and standard deviations of static and dynamic frictional force in the test and control brackets by bracket type

Bracket / Treatment	*n*	Dynamic friction*		Static friction*	
		Mean	SD	Mean	SD
Stainless-steel					
S-CHX	20	0.70	0.11	0.78	0.17
S-Control	20	0.89	0.14	0.91	0.21
Ceramic					
C-CHX	20	1.10	0.35	1.06	0.36
C-Control	20	0.99	0.35	1.06	0.42

*Two-way ANOVA: The effects of CHX treatment, bracket type or their interaction on friction were not significant.

In vitro frictional resistance results

The static and dynamic frictional forces of the metal and ceramic brackets, before and after immersion in CHX, are shown in Table 4. A two-way ANOVA was performed to examine the effect of CHX treatment and bracket type on static and dynamic frictional force. CHX had no significant effect on static frictional force ($P = 0.78$) or dynamic frictional force ($P = 0.14$). In addition, there were no significant differences between the metal and ceramic brackets in static frictional force ($P = 0.58$) or dynamic frictional force ($P = 0.82$). The interactions of CHX treatment and bracket type were also insignificant for static frictional force ($P = 0.77$) and dynamic frictional force ($P = 0.60$).

There was a difference between the topography of the metal and ceramic brackets when the slot surfaces were observed under SEM at ×2,000 magnification (Figs. 4, 5). The ceramic brackets had more smooth surfaces than the metal brackets. There were no notable differences in the images captured before and after immersion in CHX for each bracket type individually. The same observation was reported when comparing slot surfaces before and after friction tests in the test and control groups of the metal and ceramic brackets.

Discussion

Many previous studies have been designed to examine the optimum protocols for decreasing the levels of oral streptococci to control dental caries. However, few clinical studies have been performed to examine the feasibility of applying such protocols in patients treated with fixed orthodontic appliances.

CHX is one of the most popular and well-studied antimicrobial agents used in the oral cavity (14,29). Moreover, CHX has been shown to have a good safety profile (30). However, reversible adverse effects, such as impaired taste sensation, tooth staining, and occasional mucus membrane irritation, have been associated with prolonged use of CHX mouthwash (16).

A previous randomized controlled clinical trial, investigating the effectiveness of 0.50% and 0.75% CHX dentifrices in controlling gingivitis and bleeding in orthodontic patients, concluded that lower concentrations of CHX can reduce the risk of tooth staining without

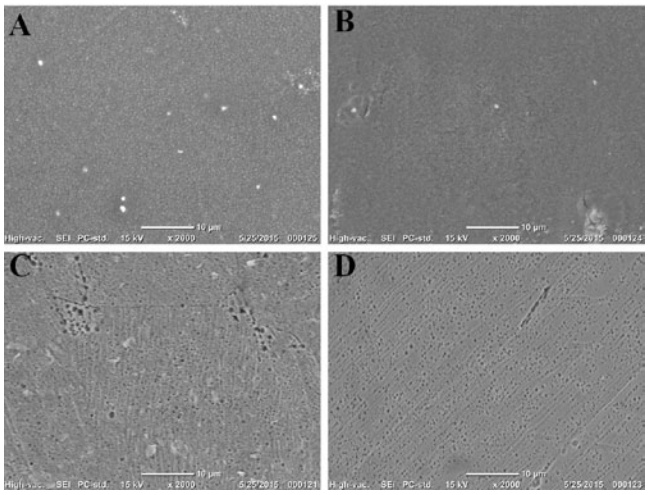

Fig. 4 Scanning electron microscopy (×2,000 magnification) images of ceramic and metal brackets before friction assessment. A, Ceramic control; B, Ceramic test; C, Metal control; D, Metal test.

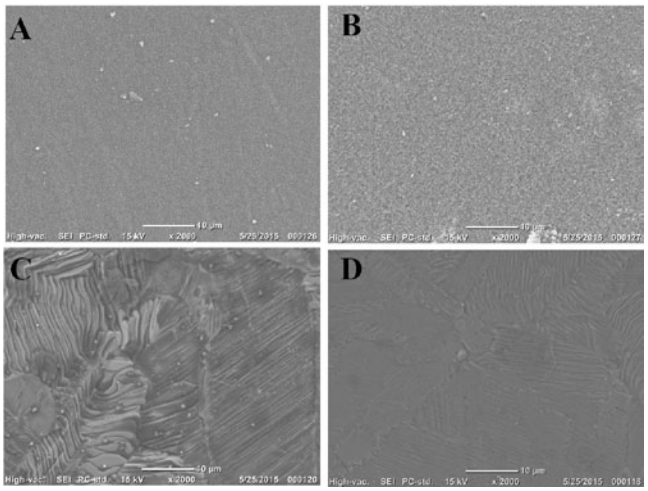

Fig. 5 Scanning electron microscopy (×2,000 magnification) images of ceramic and metal brackets after friction assessment. A, Ceramic control; B, Ceramic test; C, Metal control; D, Metal test.

compromising its effectiveness (31). The aforementioned study continued for 12 weeks, in which patients were asked to use CHX dentifrices three times daily, and the only negative side effect reported was tooth staining. However, the use of lower concentrations of CHX as dentifrices was found to be superior compared to those as mouthwash in avoiding the undesired side effects.

Different concentrations of CHX-containing mouthwashes, varnishes, and dentifrices have been tested and confirmed to reduce *S. mutans* levels in orthodontic patients. However, the long-term effects of CHX use on the oral flora and the possible adverse effects exerted on the components of fixed orthodontic appliances have not been reported (3,31). In addition, few studies have reported bacterial levels following the cessation of CHX use.

In the present study, we examined the clinical efficacy of 0.50% CHX gel, delivered twice using 3DS, in decreasing *S. mutans* in orthodontic patients. Although 3DS has been well established in the literature as an effective method of application of CHX gel in patients with high risk of caries (32-36), no clinical studies have examined the efficacy of using 3DS in orthodontic patients.

The protocol for application of CHX adopted in the current experiment did not involve the use of the drug retainer to apply fluoride gel for 5 min after brushing twice daily as previously recommended (17,18). This was conducted to avoid any potential bactericidal effects of fluoride, which may influence bacterial levels and to avoid the detrimental effects of fluoride on the titanium orthodontic wires inside patients' oral cavities (23). Despite omitting the step involving application of topical fluoride, we observed a sustained decrease in the levels of salivary *S. mutans* over the entire experimental period in our study.

The results of the current experiment provide strong evidence that PMTC was successful in removing the insoluble bacterial glucan layer, which is impermeable to several antibacterial agents (36). This step allowed higher CHX concentrations to exert potent bactericidal effects on the *S. mutans*, which was evident by the decrease in the levels of salivary streptococci observed in the current experiment. Previous research has shown that the re-colonization of microorganisms following antimicrobial agent use was because of the re-growth of existing bacteria, which had not been removed, and not because of the introduction of new bacteria (37). This may explain the sustained decrease in salivary *S. mutans* observed over the course of the experimental period in the current study.

A previous study (38) described a protocol to decrease levels of salivary *S. mutans* in an orthodontic patient with high caries risk. They reported that the level of salivary *S. mutans* was significantly decreased following the professional application of 1% CHX gel and daily mouth rinsing with 0.05% sodium fluoride solution. The results showed that the clinical remineralization of enamel was possible when 1% CHX was used in combination with daily use of a fluoride rinse. However, the small sample size used in the aforementioned study (one patient) was too small to confirm the obtained results. Moreover, the daily use of 0.05% fluoride mouthwash may have affected the levels of salivary *S. mutans* in this patient.

During the experimental period of the current study, no patients treated with the 3DS suffered from any

detrimental effects on their oral flora, which may be attributed to application of CHX in a relatively low concentration (0.50%, rather than 1 %) and application of CHX locally on the teeth with minimal contact with the saliva. The present study required no concomitant agents, such as high fluoride concentration mouth wash, which may affect the mechanical and surface properties of the components used for treating patients by fixed orthodontic appliances (23). It was previously reported that high fluoride concentrations may lead to adverse effects on the surface texture of various types of titanium-containing orthodontic wires, causing changes to their frictional resistance (23).

In the current study, we examined the possible effects of CHX gel use on the surface topography and frictional resistance of metal and ceramic brackets. The results showed that CHX use had no significant effect on the metal or ceramic brackets, which confirmed the safety of the protocol used in our study to decrease the levels of salivary *S. mutans* in patients treated with fixed orthodontic appliances.

This study was performed over a 10-week period with the final saliva sample obtained 8 weeks following the second application of CHX gel. Further investigations are recommended over a longer time period to determine the maximum extent of the effectiveness of our protocol. The use of low concentrations of CHX gel (0.50%) delivered using 3DS in combination with PMTC effectively decreased the levels of salivary *S. mutans* with no significant adverse effects on the oral environment or orthodontic brackets. Further investigations are recommended to evaluate the effect of CHX over a duration of >8 weeks, and frictional assessment should be performed using other orthodontic bracket and wire combinations.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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