

The Effects of Different Antiseptic Mouthwash On Microbiota Around Orthodontic Mini-Screw

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Research article

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Abstract

Objektives: This study aims to investigate the effects of different antiseptic mouthwash on microbiota around the mini-screw applied to patients undergoing fixed orthodontic treatment.

Materials and methods: From patients who have been undergoing fixed orthodontic treatment and who have mini-screws in their mouth, a total of 38 patients were selected for the study consisting of 4 groups, each of which has 15 mini-screws. The patients were selected from the following groups: no use of mouthwash (Group 1), use of 0.12% Chlorhexidine gluconate-containing mouthwash (Group 2), use of Essential oils-containing mouthwash (Group 3) and use of 7.5% Povidone-iodine-containing mouthwash (Group 4). Plaque indices and gingival indices of the patients were measured at the beginning (T 0) and at their appointments three weeks later (T 1). In addition, biological samples were collected from the sulcus around the mini-screw with the help of sterile paper point.

Results: The total number of microorganisms around the mini-screw in Group 2, Group 3 and Group 4 decreased significantly compared to Group 1. A significant decrease in *Streptococcus oralis* , *Streptococcus mitis* , *Candida parapsilosis*, total bacteria, plaque index and gingival index count was observed in T 1 compared to T 0 .

Conclusion: Antiseptic mouthwash in Group 2, Group 3 and Group 4 can be used to reduce the number of microbial microbiota around the mini-screw and to improve oral hygiene.

Introduction

In orthodontics, resistance to displacement and unwanted tooth movements is called anchorage [1-3]. The desired tooth movements can be achieved with the anchorage mechanics applied in orthodontics and unwanted tooth movements can be prevented [4,5]. In order to provide anchorage, many apparatuses were produced and used in orthodontics [6,7]. Among these, orthodontic mini-screws are today, the anchorage units often preferred [8].

Oral hygiene affects the success of the mini-screw. Poor oral hygiene has been found to increase the failure rate of mini-screw [9]. Apparatuses such as bands, braces and mini-screws used in orthodontic treatments create areas of plaque accumulation that feed bacteria [10]. In addition, oral hygiene cannot be maintained due to plaque accumulation caused by elastics and ligatures used [11]. Orthodontic mini-screws are open to infection due to their presence in oral microbiota. Poor hygiene of the area of the mouth where mini-screw is applied may cause inflammation between the mucosa and the screw head, which may proceed to the end of the mini-screw and lead to peri-implantitis and, thus, the loss of the mini-screw [12,13]. It has been reported that, as the hygiene of the mini-screw area increases, the risk of inflammation will decrease [14]. However, it was also stated that the hygiene of the mini-screw area is more important than oral hygiene in terms of the success of the mini-screw [15].

This study aims to investigate the effects of different antiseptic mouthwash on microbiota around the mini-screw applied to patients undergoing fixed orthodontic treatment.

Materials And Methods

Study volunteers were selected among patients who have been undergoing a fixed orthodontic treatment at the Department of Orthodontics in the Faculty of Dentistry at ... University and who have orthodontic mini-screws applied in their mouth. In the preliminary study that was undertaken before the groups were formed, the patients' daily practices that could affect their oral hygiene in daily life were investigated with open-ended questions, and since, from this data, the antiseptic mouthwash they used appeared to be more significant than other practices, it was decided that the grouping should be arranged according to the antiseptic mouthwash used.

Inclusion criteria for patients:

Patients who had no systemic diseases or allergies to any substance, who have not used any drugs or cigarettes for 1 month prior to the study and between the two sampling times, and who have a mini-screw on the posterior part of their mouth were included in the study.

Among these patients who met these criteria, 60 mini-screws in 38 patients, 20 of whom were female and 18 of whom were male, were selected for the study. 4 groups were formed with 15 mini-screws in each group. Group 1 (control group) consists of patients who do not use any mouthwash, Group 2 consists of patients who use 0.12% Chlorhexidine gluconate-containing Kloroben (Drogsan Pharmaceuticals, Ankara, Turkey) mouthwash, Group 3 consists of patients who use Essential oils (E.O.) containing Listerine (Johnson and Johnson Healthcare Products, New Jersey, USA) mouthwash and Group 4 consists of patients who use 7.5% Povidone-iodine-containing Biokadin (Kansuk Laboratory, Istanbul, Turkey) mouthwash.

All patients were given an appointment at 9:00 a.m. for the collection of samples. All patients were asked not to eat or drink anything and not to brush their teeth until the appointment time, starting from 2 hours before the appointment. Their plaque and gingival indices were measured during the first appointment (T_0). Modified Plaque Index (MPI) was used for the measurement of the plaque index and Modified Gingival Index (MGI) was used for the measurement of the gingival index. After these measurements, the sterile paper point (Ocean Endodontic Solutions, H.C.M. City, Vietnam) numbered ISO (International Organization for Standardization) 35 was placed in the sulcus around the mini-screw and we waited for 30 seconds. At the end of this period, paper points containing biological samples were removed from the sulcus and placed immediately in eppendorf tubes which contain appropriate growth mediums. Separate biological samples were collected for the eppendorf tube containing Brain-Heart Broth medium for the growth of aerobic bacteria and for the eppendorf tube containing Thioglycolate medium for the growth of anaerobic bacteria. These procedures were repeated at the next appointment 3 weeks later (T_1).

The samples were taken to a microbiology laboratory for microbiota examination immediately and incubated in an incubator at 37°C for 24 hours. After 24 hours, each medium sample was also seeded in Schaedler Agar, 5% Sheep blood medium, Eosin Methylene Blue Agar (EMB) and Sabouraud Dextrose Agar (SDA) and re-incubated. After incubation, bacterial identification and colony counts were performed on the growing bacteria using BD PHOENIX™ 100 (Becton Dickinson, Diagnostic Instrument Systems, Sparks, MD, USA).

Statistical Analysis

Statistical analyses of the results were performed using IBM SPSS for Windows (Statistical Package for Social Sciences, version: 24.0, Chicago, IL, USA) package software. The Shapiro-Wilk test was used to check the conformity of the study variables that have a continuous measurement value with normal distributions. For paired comparisons, the one-way ANOVA test was used in comparing the normal distribution compatible variable between more than two independent groups and then as a post-hoc test, LSD test was used. The Kruskal-Wallis test was used in comparing the normal distribution incompatible variables in terms of more than two independent groups and then as a post-hoc test, the Dunn test was used. Since the presumption of conformity with normal distribution was not provided for the comparison of variables with in-group continuous measurement value, The Wilcoxon Signed Ranks Test was used for the mean difference between two independent measurement times. Descriptive statistics for categorical variables are presented as n (%). The results were within the 95% confidence interval and p value <0.05 was accepted as statistically significant.

Results

The average age of 38 patients included in the study was 18.24±1.57 years. The groups were balanced and there was no statistically significant difference between the groups in terms of age ($p=0.346$) (Table 1).

Of the 38 patients included in the study, 20 (52.63%) were female and 18 (47.37%) were male. The groups were homogeneously distributed and there was no statistically significant difference between the groups in terms of gender ($p=0.966$) (Table 1).

According to the results of the microorganism culture analysis of the collected microbiota samples, a total of 12 different microorganisms were identified. At T_0 , 10 different microorganisms were detected in Group 1, and 11 different microorganisms were detected in Group 2, Group 3, and Group 4. At T_1 , 10 different microorganisms were detected in Group 1, 2 different microorganisms were detected in Group 2, 10 different microorganisms were detected in Group 3, and 8 different microorganisms were detected in Group 4. The microorganism detected at T_0 may increase, decrease at T_1 or it may never be encountered

(Table 2). When these detected microorganisms were compared at T₀ and T₁ times, there were no statistically significant difference in the number of microorganisms in Group 1 ($p=0.063$). The total number of microorganisms in Group 2, Group 3, and Group 4 decreased statistically significantly at T₁ time ($p=0.001$) ($p=0.005$) ($p=0.001$) (Table 3). In the comparison of microorganisms independent from the groups, there was a significant decrease in *S. oralis* ($p=0.007$), *S. mitis* ($p=0.012$), *C. parapsilosis* ($p=0.029$), and total bacteria count ($p<0.001$) at T₁ compared to T₀.

When the periodontal index values were compared within the group at T₀ and T₁ times, there was no statistically significant difference at the T₀-T₁ time interval for MPI, and MGI values in Group 1 ($p=0.083$) ($p=0.064$). There was a statistically significant decrease in both MPI and MGI values in Group 2 at T₁ time ($p=0.025$) ($p=0.001$). There was a statistically significant decrease in both MPI and MGI values in Group 3 at T₁ time ($p=0.001$) ($p=0.001$). In Group 4, no statistically significant difference was found between T₀-T₁ times regarding the MPI and MGI values ($p=0.180$) ($p=0.157$) (Table 4). In the comparisons between the groups, Group 2, Group 3 and Group 4 were found to be statistically significantly more successful in reducing MPI and MGI values for T₁ time ($p<0.001$). There was no statistically significant difference in the comparisons between the other groups.

Discussion

In order to provide anchorage, many apparatuses were produced and used in orthodontics [6,7]. Among these, orthodontic mini-screws are today, the anchorage units often preferred [8]. Orthodontic mini-screws are open to infection due to their presence in oral microbiota. Poor hygiene of the area of the mouth where mini-screw is applied may cause inflammation between the mucosa and the screw head, which may proceed to the end of the mini-screw and lead to peri-implantitis and, thus, the loss of the mini-screw [12,13]. Long-term maintenance is very important to reduce mini-screw losses [16]. In many studies, the ways of providing hygiene around the mini-screw and naturally reducing the loss of mini-screw have been investigated [9,11,14,15,17-20]. Poor oral hygiene has been shown as one of the important risk factors causing mini-screw losses [14,15,21-23]. Studies have shown that failed mini-screws have a microbiota similar to the one encountered in periodontal diseases [24-26]. In addition, it has been found that in the literature, studies investigating the microbiota around the orthodontic mini-screw are limited. There are many studies investigating the microbiota around the mini-screw, but there were no studies investigating the effects of different antiseptic mouthwash on this microbiota. Therefore, this study aims to investigate the effects of different antiseptic mouthwash on the microbiota around the mini-screw applied to patients undergoing fixed orthodontic treatment.

Patients receiving orthodontic treatment cannot achieve the same optimal hygiene only by brushing their teeth, as in individuals not under such treatment [27]. As orthodontic apparatuses such as bands, braces and mini-screws create areas of plaque accumulation that feed bacteria [10], oral hygiene cannot be maintained due to plaque accumulation caused by elastics and ligatures used [11]. In the literature, brushing the head of the mini-screw with a soft brush [14], cleaning the head of the mini-screw with sterile

water [15], using mouthwash [28] and antibiotic treatment [18] are some of the methods used to ensure the hygiene of mini-screw area. However, giving antibiotics to a person without any other disease just to ensure oral hygiene is a matter of debate.

In the studies carried out, the use of chlorhexidine-containing antiseptic mouthwash - which is also used by one of the groups included in our study - was recommended for patients undergoing orthodontic treatment [29-31], and for infected areas, chlorhexidine-containing mouthwash was recommended for 5-7 days [17]. Chlorhexidine demonstrates its antiseptic activity by allowing the precipitation of phosphate-containing molecules in the bacterial cell membrane [32]. Although chlorhexidine-containing mouthwash is a successful agent in preventing infections, in long-term use, it has been reported that chlorhexidine may cause discoloration of the tongue and teeth [33-35], taste changes in mouth [33,34,36], and desquamation and irritations in the soft tissues of the mouth [33,37-40]. E. O. containing mouthwash used by another group in our study are formulated by mixing essential oils with menthol, thymol, eucalyptol and methyl salicylate. They demonstrate bactericidal effect by denaturing porins in the bacterial cell membrane and leaking low molecular weight molecules in cytoplasm out of the cell [41]. In a study, the efficacy of E.O. containing antiseptics against a broad spectrum of bacteria was demonstrated, but it was reported that the oral microbiota was suppressed in the first hours after application [42]. Chemotherapeutics containing E.O. and chlorhexidine are effective even against bacteria embedded in the biofilm by penetrating into the biofilm layer [43-45]. Antiseptics containing substances such as manuka oil and tea tree oil that are in the E.O. group have been reported to have bactericidal effects against peri odontogenic and cariogenic bacteria such as *P. gingivalis* and *S. mutans* [46], prevent plaque formation and gingivitis [47], and it has also been reported that Listerine mouthwash containing E.O. used in patients undergoing orthodontic treatment causes a significant decrease in MPI and MGI [48,49] and may be recommended for patients undergoing orthodontic treatment [48]. The 7.5% Povidone-iodine which is the antiseptic mouthwash used by another group in our study is the most commonly used iodine derivative antiseptic. At a concentration of 1/200.000, it kills all vegetative forms of bacteria within 15 minutes. They demonstrate effective bactericidal, fungicidal, virucidal and sporicidal effects even at low concentrations. They cause high irritation and staining [50-52]. They demonstrate bactericidal effect by affecting the main protein groups, nucleotides, and fatty acids by disrupting the electron transport of iodine aerobic microorganisms penetrating into the microorganism cell wall with an oxidative effect [53].

The sterile paper point that we use to collect microbial samples is used in endodontic treatments in dentistry and in the collection of biological samples in small areas in medicine. In a study whereas biological sample was collected with a paper point, it was stated that the paper point showing the number of bacteria in the test medium most accurately was ISO 45 and that 30- or 60-second periods can be used for sample collection [54]. In another study where sample was collected from the mini-screw area, ISO 35 paper point was used [20]. However, this study did not mention in how many seconds the samples were taken. In another study carried out in the mini-screw area, ISO 45 paper point was used and this study also did not mention in how many seconds the samples were taken [19]. In the preliminary clinical evaluation before our study, it was observed that the paper points numbered 45 and 40 caused bleeding in the mucosa, and that the paper points 30 and below could not remain stable for 30 seconds

and dropped due to the liquid absorbed. Considering all these reasons, sampling time was determined as 30 seconds and ISO 35 paper point was chosen for sampling.

Streptococcus bacteria are the most common bacteria encountered in the studies investigating the microbiota in the mini-screw area [19,20,55]. This may be due to the fact that these bacteria are also present in the normal oral microbiota. In our study, among the 12 species of microorganisms detected, 7 of them were streptococcus. In this respect, the results are similar to the literature. In our study, 6 different growth mediums were used to detect all bacteria that can be cultured. The aim was to produce the maximum number and variety of microorganisms possible. Cultivation and detection of such a wide spectrum of microorganisms is one of the superior aspects of the study.

The effects of different antiseptic mouthwash on microbiota around the mini-screw applied to patients undergoing fixed orthodontic treatment were investigated in this study and the following results were obtained:

1. Brushing teeth only is not sufficient to maintain hygiene and reduce microbiota around the mini-screw in patients undergoing orthodontic treatment.
2. Antiseptic mouthwash containing 0.12% Chlorhexidine, Essential oils (E.O.) and 7.5% Povidone-iodine were found to be successful in reducing the microbiota around the mini-screw.
3. In order to avoid the undesirable effects of these agents, it is considered that more comprehensive studies should be done clinically with larger patient groups in order to see the effects of combined use at different times and periods.

Compliance Of Ethical Standards

Conflict of Interest: Author Y. A. declares that he has no conflict of interest.

Funding: The study was not supported by any institution.

Ethical approval: During the course of this study, the ethical principles of medical research on human volunteers specified in the World Medical Association (WMA) Declaration of Helsinki were adhered to. Ethics Committee approval dated 24/01/2019 with meeting no. 02 and decree no. 10 was obtained from ... University Ethics Committee for the research.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1: Distribution of age and sex by groups

	Group 1		Group 2		Group 3		Group 4		Total		p
Age	n	mean±SD	n	mean±SD	n	mean±SD	n	mean±SD	n	mean±SD	0.346
	10	17,71 ± 1,57	9	18,58 ± 1,93	9	18,07 ± 1,51	10	18,60 ± 1,15	38	18,24 ± 1,57	
Gender	n	%	n	%	n	%	n	%	n	%	0.966
Female	5	50,0	5	55,6	5	55,6	5	50,0	20	52,63	
Male	5	50,0	4	44,4	4	44,4	5	50,0	18	47,37	

Table 2: Number of microorganisms detected in groups

	Microorganisms (Cfu/ml)	T ₀ (mean±SD)	T ₁ (mean±SD)	p
Group 1	<i>S. mitis</i>	48000±47689	57330±48912	0,059
	<i>S. oralis</i>	37330±41998	46670±45774	0,109
	<i>S. viridans</i>	5330±20656	5330±20656	1,000
	<i>S. mutans</i>	6670±25820	5330±20656	0,317
	<i>S. salivarius</i>	6670±25820	6670±25820	1,000
	<i>S. gordonii</i>	6670±25820	6670±25820	1,000
	<i>S. pyogenes</i>	5330±20656	5330±20656	1,000
	<i>S. aereus</i>	6670±25820	6670±25820	1,000
	<i>S. epidermidis</i>	12000±31893	17330±36148	0,317
	<i>C. albicans</i>	14670±31593	18670±32484	0,461
Group 2	<i>C. parapsilosis</i>	0	0	1,000
	<i>E. coli</i>	0	0	1,000
	<i>S. mitis</i>	44000±48961	13330±27946	0,018*
	<i>S. oralis</i>	50670±45898	13330±23503	0,007*
	<i>S. viridans</i>	6000±23238	0	0,317
	<i>S. mutans</i>	4000±15492	0	0,317
	<i>S. salivarius</i>	6670±25820	0	0,317
	<i>S. gordonii</i>	24000±42224	0	0,059
	<i>S. pyogenes</i>	0	0	1,000
	<i>S. aereus</i>	13330±35187	0	0,157
Group 3	<i>S. epidermidis</i>	14670±35024	0	0,102
	<i>C. albicans</i>	15330±29968	0	0,068
	<i>C. parapsilosis</i>	22670±39905	0	0,066
	<i>E. coli</i>	4000±11212	0	0,180
	<i>S. mitis</i>	40000±50709	33330±42538	0,025*
	<i>S. oralis</i>	46670±46394	38670±43072	0,257
	<i>S. viridans</i>	5330±20656	5330±20656	1,000
	<i>S. mutans</i>	6670±25820	5330±20656	0,317
	<i>S. salivarius</i>	6670±25820	5330±20656	0,317
	<i>S. gordonii</i>	6670±25820	5330±20656	0,317
Group 4	<i>S. pyogenes</i>	0	0	1,000
	<i>S. aereus</i>	6670±25820	5330±20656	0,317
	<i>S. epidermidis</i>	6670±25820	6670±20931	1,000
	<i>C. albicans</i>	18670±31593	8000±22424	0,039*
	<i>C. parapsilosis</i>	6670±25820	0	0,317
	<i>E. coli</i>	4000±11212	1330±5164	0,414
	<i>S. mitis</i>	67330±43991	14670±31593	0,005*
	<i>S. oralis</i>	43330±40999	2670±10328	0,007*
	<i>S. viridans</i>	5330±20656	0	0,317
	<i>S. mutans</i>	4000±15492	2670±10328	0,317

	<i>C. parapsilosis</i>	0	0	1,000
	<i>E. coli</i>	1330±5164	2000±5606	0,317

Table 3: Average number of microorganisms in the groups at T₀ and T₁ times

Total number of microorganisms (Cfu/ml)	T ₀ (mean±SD)	T ₁ (mean±SD)	p
Group 1	149.330±77.779	176.000±68.536	0,063
Group 2	205.330±69.268	26.670±30.861	0,001*
Group 3	153.330±79.522	116.000±68.951	0,005*
Group 4	175.330±48.087	38.000±51.158	0,001*

Table 4: In-group distributions of periodontal index mean values

Groups	MPI	mean±SD	p	MGI	mean±SD	p
Group 1	T ₀	1,93 ± 0,59	0,083	T ₀	1,60 ± 0,74	0,064
	T ₁	2,13 ± 0,64		T ₁	1,87 ± 0,83	
Group 2	T ₀	1,47 ± 0,52	0,025*	T ₀	1,33 ± 0,49	0,001*
	T ₁	1,13 ± 0,35		T ₁	0,33 ± 0,49	
Group 3	T ₀	1,93 ± 0,59	0,001*	T ₀	1,60 ± 0,73	0,001*
	T ₁	1,00 ± 0,00		T ₁	0,67 ± 0,48	
Group 4	T ₀	1,87 ± 0,64	0,180	T ₀	1,40 ± 0,51	0,157
	T ₁	1,67 ± 0,49		T ₁	1,27 ± 0,46	

Figures



Figure 1

Placement of paper point between the head of mini-screw and mucosa