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The effect of the octenidine-based oral antiseptic on the structure of microbial communities and periodontal status in patients with fixed orthodontic treatments

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Abstract. – OBJECTIVE: To evaluate the effect of an octenidine (OCT)-based antiseptic on the gingival inflammation and microbial composition of subgingival dental plaque in patients with fixed orthodontic appliances.

PATIENTS AND METHODS: Thirty-three orthodontic patients were randomized into 2 groups. The control group patients were given standard oral hygiene and dietary advice, while the experimental group patients used an OCT-based antiseptic together with standard oral hygiene and dietary recommendations. The periodontal status was evaluated using the following indices: the plaque index (PI), the gingival index (GI), the papilla bleeding index (PBI) and the probing pocket depth (PD). Next Generation Sequencing of the 16S rRNA amplicons was performed in order to assess the subgingival microbiome.

RESULTS: The PD values obtained were significantly lower in the experimental group after one month, as well as PBI. The microbiological analysis showed a significant increase in the occurrence of the genus *Prevotella* in the control group, while the number of other periodontopathogens remained stable in both groups. The changes in the abundance of the bacteria not directly associated with periodontal disease were also observed.

CONCLUSIONS: The use of an OCT-based antiseptic has a positive effect on the prevention of gingival inflammation. Additionally, it also prevents a likely increase in numbers of periodontopathogens of the subgingival dental plaque in the first three months of fixed orthodontic treatment.

Key Words:
Orthodontics, Oral antiseptic, Periodontal status, Microbial communities.

Introduction

A fixed orthodontic treatment is used to correct malocclusion and deformities in order to

improve oral health and dental aesthetics. However, placement of orthodontic brackets and bands can make oral hygiene difficult, leading to the accumulation of dental plaque, thus increasing the risk of caries[1,2] and periodontal disease[3-5]. The results[6-10] previously published show an association of orthodontic treatments with unfavorable quantitative and qualitative microbial changes of subgingival dental plaque leading to a development of gingivitis and periodontitis. Oral antiseptic solutions can contribute to better control of dental plaque[11,12]. One of the most commonly used antiseptics is chlorhexidine (CHX). Although CHX is very effective in reducing dental plaque, its use is limited due to side effects, such as tooth staining, build-up of calculus, temporary taste disturbance, and changes in the oral mucosa[13]. In addition, it is readily deactivated by common ingredients of toothpastes, in particular, anionic surfactants[14]. Hence, other antiseptics comparable to CHX in terms of antiseptic activity and inhibition of accumulation of biofilms are being investigated. Octenidine hydrochloride (OCT) is a bispyridine derivative with broad antimicrobial specter[13]. It has been used for more than 30 years as a 0.1% oral solution significantly reducing plaque accumulation and gingivitis, thus improving periodontal health[15,16]. The published research data evaluating the effects of fixed orthodontic

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treatments on the periodontium and subgingival microflora are inconsistent. Most of the research conducted so far analyzed the frequency of periodontopathogens in subgingival plaque. However, none of the reported studies used the next generation sequencing technique to analyze the complete subgingival microbiome and its quantitative and qualitative changes during fixed orthodontic treatments. Furthermore, none of the published studies deal with combined effects of OCT-based antiseptics on both periodontal clinical parameters and subgingival microbial composition in orthodontic patients. The aim of this study was to evaluate the clinical conditions of periodontium and composition of the complete subgingival microbiome during fixed orthodontic appliance placement, as well as to observe the effect of the use of OCT-based antiseptic in comparison with standard dental hygiene protocol (without OCT) one and three months after the treatment.

Patients and Methods

Periodontal Status Assessment

The subjects of the study were orthodontic patients of both sexes with an indication for fixed orthodontic appliance placement. The exclusion criteria were: diagnosed periodontal disease, the use of antibiotics in the last 3 months, a presence of systemic disease that can affect the condition of the periodontium. All patients or their parents, if they were younger than 18 years signed the informed consent form. All patients underwent an initial assessment of the periodontal conditions. The following indices were used: the plaque and the gingival index Silness-Löe (PI)[17], the papilla bleeding index − Mühlemann (PBI)[18] and the probing depth (PD) using a calibrated periodontal probe (UNC-15, Hu Friedy, Chicago, III, IL, USA). All periodontal parameters were assessed at four sites (mesio-buccal, mid-buccal, distobuccal, and mid-lingual) of each tooth present, excluding 3rd molars. A full periodontal examination was performed by a single examiner followed by plaque sampling for microbiological analysis. The subgingival plaque was collected from mesio-buccal gingival crevice of the right upper first molar by placing a sterile curette into the bottom of the sulcus and drawing it coronally with slight pressure against the tooth. Each sample was wiped off on to a swab and placed into plastic tube with Isohelix Dri-Capsules (https://isohelix.com/products/isohelix-dna-buccal-swabs/, Cell Projects Limited, Harrietsham, Kent, UK). If the first molar was missing, the surface of the contralateral or adjacent tooth was used instead. The sampling sites were isolated from saliva with cotton roles and gently dried with air.

All patients were instructed to use the Bass brushing technique and were given dietary recommendations. Patients were randomized into two groups: the experimental and the control group. The experimental group patients used an OCT-based antiseptic (15 ml two times a day, 7 days the first week of the month during the study period) in addition to the recommended oral hygiene protocol. The control group used standard oral hygiene and dietary protocol only. Periodontal condition was reassessed and subgingival plaque samples were collected from the same site as the initial sample one and three months after the placement of fixed orthodontic appliances.

DNA Extraction, Sequencing and Sequence Analyses

Ninety-nine samples were collected into Isohelix tubes. Five samples could not be analyzed due to damages that occurred during technical preparation. The DNA from the 94 samples collected by Isohelix SK-1S buccal swabs was extracted using the Isohelix BEK-50 Single Step/Single Tube DNA Release Kit according to the manufacturer's instructions (https://isohelix.com/, Cell Projects Limited, Harrietsham, Kent, UK). Quantity of the DNA was assessed using a Qubit 2.0 fluorometer (Life technologies Ltd, Paisley, UK). The next Generation Sequencing of 16S rRNA amplicons was performed on an Illumina MiSeq PE250 instrument; San Diego, CA, USA) using the 515F – 806R 16S rRNA primers (Earth Microbiome Project) targeting the V4 region at the Finnish Institute for Molecular Medicine, Helsinki, Finland. A total of 13 million sequences were generated averaging a sequencing depth per sample of 144,000 reads. Taxonomic classification was performed using the Illumina BaseSpace app 16S Metagenomics against the Illumina-curated version of the GreenGenes database.

Statistical Analysis

Data were analyzed using the SPSS 20.0 software (IBM Armonk, NY, USA) expressed as mean ± SD, median with IQR (interquartile range) or numbers (%), depending on the distribution and type of data. If distribution was normal, or if it could be normalized by transforming the data, the *t*-test was used in order to assess the differences between the variables. The non-parametric

approaches were used for skewed variables. The differences between variables were assessed by the Mann-Whitney U test and the Friedman's test if distribution was non-normal. Categorical variables were compared using the Chi-square test. The *p*-value (statistical significance) was set at 0.05 using the Bonferroni correction, if required. Additional data are shown in the **Supplementary file.**

Results

Thirty-three patients participated in the study (7 males and 26 females; mean age 19.85 years). The experimental (N=22) and the control group (N=11) did not differ significantly regarding age, gender, and whether they followed recommended oral hygiene and dietary protocol throughout the period of the study.

Initially, the statistically significant differences of the periodontal indices were not observed between the groups. However, one month after the placement of fixed orthodontic appliances, the PD values were significantly lower in the experimental group while a decrease in the PBI values was observed in the control group three months after the placement of the appliances (Table I).

The mean total number of the genera identified in samples by the Illumina NGS sequencing was 110 (minimum 32, maximum 228, three samples per patient, 94 samples in total). A statistical analysis of the 8 most abundant bacterial genera per sample as reported by the Illumina BaseSpace app 16S Metagenomics was performed totaling 39 bacterial genera from all samples. The data were subjected to statistical analyses. The presented results are the percentages of reads of the 39 bacterial genera of the total number of reads of all identified taxa (110 genera). (Table II and Table III).

Moraxella, *Renibacter*, *Variovorax*, Candidatus-*Tammella* were also identified among the 39 top classifications. However, since they made less than 0.01% (median) of the total number of reads, these taxa were not included in the tables.

Discussion

The published data evaluating the effects of fixed orthodontic treatments on periodontium (plaque accumulation and periodontal diseases) are inconsistent[8,19,20]. It is well known that patients tend to be highly motivated to follow the hygiene maintenance instructions right after the visit to the dentist. The initial PI values, as well as other clinical parameters in the study, were assessed during the same appointment when patients were advised on how to maintain oral hygiene. In addition, the patients were probably additionally motivated to improve brushing technique after being informed that a plaque sampling was going to be performed during the control check-ups. Other studies also confirmed high impact of motivation of orthodontic patients on improvement of oral hygiene[21,22].

Table I. Periodontal indices of the two groups.

		Time					
		T1		T2		T3	
		Mean ±SD	*p*	Mean±SD	*p*	Mean±SD	*p*
PI	Control group Experimental group	0.64±0.26 0.86±0.48	0.22	0.66±0.33 0.67±0.43	0.92	0.66±0.34 0.62±0.43	0.71
GI	Control group Experimental group	0.65±0.3 0.73±0.33	0.66	0.60±0.23 0.58±0.24	0.62	0.57±0.17 0.56±0.34	0.72
PBI	Control group Experimental group	0.94±0.26 0.92±0.51	0.9	0.91±0.38 0.64±0.4	0.75	0.83±0.29 0.58±0.19	**<0.01***
PD	Control group Experimental group	1.63±.19 1.63±0.23	0.97	1.83±0.2 1.61±0.16	**<0.01***	1.82±0.23 1.73±0.19	0.22

(*t*-test); SD-standard deviation; *p*-statistical significance; *$p \leq 0.05$; PI/plaque index; GI/gingival index; PBI/papilla bleeding index; PD/probing depth; T1/initial values of indices; T2/values of indices one month after fixed orthodontic appliance placement; T3/values of indices 3 months after fixed orthodontic appliance placement.

Table II. The differences between the percentages of the 39 most abundant genera identified based on the total number of reads in three-time intervals (initially, one month after fixed orthodontic appliance placement and three months after fixed orthodontic appliance placement).

Identified bacteria	Control group				Experimental group			
	T1	T2	T3	*p*	T1	T2	T3	*p*
Kingella%	0.17	0.11	0.28	0.97	0.22	0.86	0.97	**0.02***
Veionella%	11.19	6.01	15.21	**0.01***	12.92	10.43	20.63	0.45
Parascardovia%	4.77	2.45	3.24	0.46	5.82	3.45	2.31	0.35
Neisseria%	4.53	2.06	2.62	0.72	3.55	1.72	1.52	0.09
Hemophilus%	0.23	0.13	0.12	0.92	1.01	0.29	0.61	**0.02***
Lautropia%	1.23	1.04	0.91	0.72	7	2.68	3.82	**0.04***
Corynebacterium%	0.76	1	2.57	0.64	1.27	0.68	1.02	0.21
Cardiobacterium%	0.55	0.86	1.26	0.17	1.04	1.37	1.26	0.52
Conchiformibius%	0.01	0	0.01	0.61	0.02	0.26	0.12	0.37
Selenomonas%	8.06	28.83	12.56	0.12	3.40	6.14	15.84	**0.01***
Fusobacterium%	**4.66**	**6.64**	**7.25**	**0.37**	**2.94**	**3.67**	**4.41**	**0.52**
Capnocytophaga%	2.06	1.95	1.75	0.37	1.35	2.44	1.83	0.4
Streptococcus%	4.60	3.89	3.87	0.64	1.54	2.15	1.39	0.52
Actinomyces%	**0.53**	**0.52**	**0.68**	**0.92**	**0.49**	**0.58**	**0.43**	**0.79**
Rothia%	0.32	0.28	0.57	1.0	0.68	4	0.54	0.76
Sphingomonas%	1.20	0.78	0.81	0.89	0.41	0.25	0.12	**0.05***
Pectinatus%	0.16	0.51	0.80	0.46	0.04	0.33	0.32	0.13
Campylobacter%	0.70	0.67	1.18	0.45	0.55	0.90	1.04	0.25
Megasphaera%	0.15	0.02	0.07	0.81	0.01	0.03	0.13	**<0.01***
Gemella%	1.42	0.68	0.92	0.46	0.13	0.14	0.13	0.34
Prevotella%	**0.96**	**2.76**	**2.70**	**0.04***	**0.73**	**1**	**1.53**	**0.16**
Leptotrichia%	1.64	1.56	1.74	0.89	0.35	0.49	0.54	0.23
Actinobacillus%	0.04	0.02	0.01	0.8	0.09	0.03	0.06	**0.02***
Aggregatibacter%	**0.33**	**0.71**	**0.65**	**0.69**	**0.25**	**0.25**	**0.18**	**1.0**
Curvibacter%	0.12	0.06	0.10	0.44	0.10	0.10	0.07	0.17
Unclassified bacteria%	1.40	1.25	2.92	0.26	0.76	0.84	0.77	0.86
Eikenella%	0.32	0.29	0.12	**0.02***	0.20	0.23	0.12	0.42
Abiotrophia%	0.51	0.04	0.01	0.1	0.08	0.01	0.01	**<0.01***
Porphyromonas%	**0.44**	**0.33**	**0.19**	**0.89**	**0.06**	**0.16**	**0.05**	**0.23**
Tannerella%	**0.21**	**0.38**	**0.41**	**0.49**	**0.04**	**0.05**	**0.06**	**0.69**
Dialister%	0.06	0.10	0.21	0.42	0.01	0.02	0.05	0.17
Sphingobacterium%	0.07	0.33	0.08	0.43	0	0.01	0	**0.03***
Phyllobacterium%	0.11	0.02	0.04	0.27	0	0	0	1.0
Chryseobacterium%	0.32	0.22	0.35	0.82	0.10	0.08	0.12	0.14
Atopobium%	0.01	0.05	0.04	0.51	0	0.01	0.02	**0.01***

(*t*-test); SD-standard deviation; *p*-statistical significance; *$p \leq 0.05$; PI/plaque index; GI/gingival index; PBI/papilla bleeding index; PD/probing depth; T1/initial values of indices; T2/values of indices one month after fixed orthodontic appliance placement; T3/values of indices 3 months after fixed orthodontic appliance placement.

Table III. The differences between percentages of the 39 most abundant genera identified based on the total number of reads in three-time intervals (initially, one month later, and three months later) for control and experimental group.

Identified bacteria	T1			T2			T3		
	Control group	Experimental group	*p*	Control group	Experimental group	*p*	Control group	Experimental group	*p*
Kingella%	0.17	0.22	0.37	0.11	0.86	**<0.01***	0.28	0.97	**0.01***
Veionella%	11.19	12.92	0.46	6.01	10.43	0.12	15.21	20.63	0.71
Parascardovia%	4.77	5.82	0.74	2.45	3.45	0.26	3.24	2.31	0.96
Neisseria%	4.53	3.55	0.65	2.06	1.72	0.55	2.62	1.52	0.37
Hemophilus%	0.23	1.01	**0.04***	0.13	0.29	**0.03***	0.12	0.61	**0.05***
Lautropia%	1.23	7	**0.04***	1.04	2.68	**0.02***	0.91	3.82	0.35
Corynebacterium%	0.76	1.27	0.50	1	0.68	0.53	2.57	1.02	0.26
Cardiobacterium%	0.55	1.04	0.23	0.86	1.37	0.63	1.26	1.26	0.87
Conchiformibius%	0.01	0.02	0.13	0	0.26	**<0.01***	0.01	0.12	**0.02***
Selenomonas%	8.06	3.40	0.12	28.83	6.14	**0.02***	12.56	15.84	0.71
Fusobacterium%	**4.66**	**2.94**	**0.35**	**6.64**	**3.67**	**0.06**	**7.25**	**4.41**	**0.19**
Capnocytophaga%	2.06	1.35	0.95	1.95	2.44	0.92	1.75	1.83	0.96
Streptococcus%	4.60	1.54	0.01*	3.89	2.15	**0.05***	3.87	1.39	**<0.01***
Actinomyces%	**0.53**	**0.49**	**0.97**	**0.52**	**0.58**	**0.86**	**0.68**	**0.43**	**0.48**
Rothia%	0.32	0.68	0.25	0.28	4	0.43	0.57	0.54	0.64
Sphingomonas%	1.20	0.41	**0.03***	0.78	0.25	**0.03***	0.81	0.12	**0.01***
Pectinatus%	0.16	0.04	0.06	0.51	0.33	0.20	0.80	0.32	0.20
Campylobacter%	0.70	0.55	0.97	0.67	0.90	0.95	1.18	1.04	0.59
Megasphaera%	0.15	0.01	0.18	0.02	0.03	0.97	0.07	0.13	0.69
Gemella%	1.42	0.13	**0.01***	0.68	0.14	**0.01***	0.92	0.13	**<0.01***
Prevotella%	**0.96**	**0.73**	**0.26**	**2.76**	**1**	**0.07**	**2.70**	**1.53**	**0.08**
Leptotrichia%	1.64	0.35	0.10	1.56	0.49	**<0.01***	1.74	0.54	0.23
Actinobacillus%	0.04	0.09	**0.01***	0.02	0.03	0.23	0.01	0.06	0.06
Aggregatibacter%	**0.33**	**0.25**	**0.54**	**0.71**	**0.25**	**0.07**	**0.65**	**0.18**	**0.52**
Curvibacter%	0.12	0.10	0.71	0.06	0.10	0.14	0.10	0.07	0.76
Unclassified bacteria%	1.40	0.76	**0.01***	1.25	0.84	0.06	2.92	0.77	**0.01***
Eikenella%	0.32	0.20	0.38	0.29	0.23	0.19	0.12	0.12	0.78
Abiotrophia%	0.51	0.08	0.07	0.04	0.01	0.08	0.01	0.01	0.75
Porphyromonas%	**0.44**	**0.06**	**0.03***	**0.33**	**0.16**	**0.28**	**0.19**	**0.05**	**0.07**
Tannerella%	**0.21**	**0.04**	**0.37**	**0.38**	**0.05**	**0.02***	**0.41**	**0.06**	**0.05***
Dialister%	0.06	0.01	0.37	0.10	0.02	0.42	0.21	0.05	0.24
Sphingobacterium%	0.07	0	0.13	0.33	0.01	0.06	0.08	0	0.06
Phyllobacterium%	0.11	0	**<0.01***	0.02	0	**<0.01***	0.04	0	**<0.01***
Chryseobacterium%	0.32	0.10	0.06	0.22	0.08	**0.01***	0.35	0.12	**0.05***
Atopobium%	0.01	0	0.49	0.05	0.01	0.28	0.04	0.02	0.43

(Mann-Whitney U test, Data are presented as median); *p*-statistical significance; *$p \leq 0.05$; T1/initial values of indices; T2/values of indices one month after fixed orthodontic appliance placement; T3/values of indices 3 months after fixed orthodontic appliance placement.

Additional use of oral antiseptics usually offers advantages in controlling the formation of dental plaque and prevention of gingivitis[23]. The OCT-based antiseptic used in this study contributed to prevention of gingival inflammation since the PBI and PD values one month after the treatment were significantly lower in the OCT using group of patients when compared to the control group. The papilla bleeding index, one of the most relevant indicators of gingival inflammation, remained significantly lower during the three-month check-up in accordance with previously published results by Beiswanger et al[24].

One of the aims of the study was to determine microbial composition of subgingival dental plaque in patients with fixed orthodontic treatment initially, one and three months after appliances placement, as well as the effects of an OCT-based antiseptic on the composition of the plaque. In order to profile the subgingival microbiome, we used the Next Generation Sequencing of the 16S rRNA amplicons, a metagenomics approach that has not been typically used in the past. The chosen approach has revealed a complete microbial composition of subgingival samples including the non-cultivable members of the community. The mean number of the identified genera in each sample was 110 (minimum 32, maximum 228). The 39 most abundant genera were subjected to a statistical analysis.

Veillonela was the dominant genus detected in the initial samples of both groups of patients. *Veillonela parvula* is considered a useful and protective member of the genus, since it prevents colonization and proliferation of pathogenic microorganisms. It is usually located on sites that do not show signs of periodontal destruction and attachment loss[25]. The identified predominance of the genus among the patients indicates a good periodontal health, which remained at the same level throughout the study. Importantly, an increase in *Veillonela* numbers was more pronounced among the patients who used the OCT-based antiseptic.

A special attention was paid to the abundance of genera *Porphyromonas, Aggregatibacter, Tannerella,* and *Prevotella*, since they include the most significant periodontopathogens, such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia,* and *Prevotella intermedia*.

The *Prevotella* genus was the most abundant (0.96% in the control group and 0.73% in the experimental group) increasing significantly one and three months after the placement of fixed orthodontic appliances in the control group of patients. A statistically insignificant increase in the *Prevotella* numbers was also observed in patients who used the oral antiseptic. Kim et al[19] have also reported the significant increase of genus *Prevotella* in subgingival plaque after the placement of fixed orthodontic appliances, which remained high even after 6 months. Guo et al[7] reported a statistically insignificant increase of *Prevotella* species one and three months after placement of fixed orthodontic appliances. Zivkovic-Sandic et al[26] detected the rising trend of *Prevotella* in 15 patients one month after fixed orthodontic treatment with a declining trend after three months.

Aggregatibacter actinimycetmcomitans, as one of the most aggressive periodontopathogens was present in 0.33% of the reads in the control group and 0.25% in the experimental group in the initial samples collected from the patients. An increasing trend of the presence of the genus in the control group was observed, while its levels declined in patients using the OCT-based antiseptic, in accordance with the results published by Guo et al[7] observing the increasing trend during fixed orthodontic treatments. The results obtained by Paolantonio et al[27] showed an initial absence of the pathogen followed by a subsequently isolation from 20 subjects 8 weeks after placement of fixed appliances.

The counts of *Porphyromonas* spp. did not change significantly after the placement of fixed orthodontic appliances which is in accordance with the results obtained by Kim et al[19]. To the contrary, Liu et al[28] detected significant decrease of the pathogen in orthodontic patients. In addition, our results do not show a correlation between changes in the *Porphyromonas* counts and the general periodontal status.

A low presence of the genus *Tannerella* which includes the putative pathogen *Tannerella fosythia* was detected. Its abundance increased in patients who used the standard protocol of oral hygiene only, while remaining unchanged in patients using the OCT-based antiseptic. The significantly higher *Tannerella* counts were observed in the control group one and three months after fixed orthodontic treatment which is in accordance with previously published data[7,9,19].

Generally, we observed a greater biodiversity throughout the study among patients using the OCT-based antiseptic, namely an increase in numbers of *Kingella, Hemophilus, Selenomonas, Megasphera,* and *Atopobium* with a reduction of *Lautropia*. *Kingela* and *Selenomonas* usually present at sites affected by periodontitis. Moreover, we observed increasing levels of the putative periodontopathogen *Prevotella* and a decrease of *Eikenella* counts in the control group[29].

Conclusions

According to the results obtained, among the patients who followed the standard oral hygiene protocol, a generally lower periodontal status has been determined, including the increased levels of periodontopathogens of the genera *Prevotella* and *Tanerella* when compared to the patients additionally using the OCT-based antiseptic. On average, 110 bacterial genera were detected per sample with a trend of an increasing biodiversity in the experimental group. Initially, *Veillonela* was the dominant member of the microbiome among all subjects. The counts of the members of the *Prevotella* genus were significantly elevated in the control group over the course of the study. In addition, an increased presence of *Tannerella* was detected in the control group while remaining stable in patients using the oral antiseptic. Thus, based on the data obtained, the use of an OCT-based antiseptic had a positive effect on the prevention of gingival inflammation, as well as a decrease of periodontopathogens in subgingival dental plaque, during the first three months after the placement of fixed orthodontic appliances.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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