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## ORIGINAL ARTICLE

# Influence of Cervitec gel on periodontal health of patients wearing fixed orthodontic appliances

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## KEYWORDS

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*Porphyromonas  
gingivalis*;  
*Treponema denticola*

**Abstract** *Background/purpose:* This double-blinded randomized placebo-controlled study aimed to evaluate the efficacy of 0.2% chlorhexidine containing Cervitec gel on periodontal health during orthodontic treatment.

*Materials and methods:* Twenty-five patients undergoing fixed orthodontic treatment were randomly assigned to the Cervitec ( $n = 13$ ) or control ( $n = 12$ ) groups. After clinical examination at first visit, all patients received professional prophylaxis, and 2 weeks later baseline (B) evaluations are performed. Later, oral hygiene procedures were refrained for 3 days until Day 0, during which the participants in the Cervitec group were instructed to brush with standard toothpaste (ST) ( $1 \times 1$ ) and Cervitec ( $1 \times 1$ ), whereas the control group received placebo ( $1 \times 1$ ) until Day 14. Between Day 14 and Day 28, patients returned to brushing with ST ( $2 \times 1$ ). The clinical measurements were recorded and subgingival plaque samples were collected at first visit, B, Day 0, Day 14, and Day 28. Subgingival plaque samples were analyzed for total bacteria, *Porphyromonas gingivalis* (P.g.), and *Treponema denticola* (T.d.) using real-time polymerase chain reaction. The data were statistically analyzed.

*Results:* After receiving professional prophylaxis and oral hygiene instructions, remarkable improvements was seen in clinical and microbiological variables of the study. Although there was a significant reduction in the Quigley–Hein Plaque Index (mQHI) score at Day 14 in only the Cervitec group ( $P < 0.01$ ), both the Cervitec group and the control group revealed significant reductions in the mQHI score at Day 28 compared with Day 0 ( $P < 0.001$  and  $P < 0.01$ , respectively). Intragroup and intergroup evaluations revealed no statistically significant alterations for P.g. and T.d.

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**Conclusion:** The present data suggested that brushing with Cervitec gel once a day has the potential to reduce bacterial accumulation around teeth and fixed appliances in patients undergoing orthodontic treatment. However, within the limits of this study, Cervitec seems to have no significant effect on total bacteria, P.g., and T.d. levels of subgingival dental plaque.

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## Introduction

Maintaining a good or acceptable oral hygiene is a difficult task for patients undergoing orthodontic treatment with fixed appliances. Previously, fixed orthodontic appliances were associated with the development of white-spot lesions and impaired periodontal health due to increased dental plaque accumulation around appliances such as bands, braces, archwire ligation, and elastomeric ring.<sup>1,2</sup> The rough surfaces and the presence of distinct gaps around the bracket bases are reported to be critical sites for bacterial plaque accumulation.<sup>1</sup> Furthermore, following tooth banding, increase of pocket probing depths (PDs), decrease of anaerobe-to-facultative bacteria ratio, and increase of black-pigmented bacteroides, *Bacteroides intermedius* (*Prevotella intermedia*), and *Actinomyces odontolyticus* species were reported.<sup>2</sup> It was suggested that regular advices and routine instructions in oral and fixed appliance hygiene given to this group of patients are not sufficient and did not completely overcome the possible detrimental effects of plaque accumulation.<sup>3</sup>

Chlorhexidine digluconate (CHX) is a well-known cationic bisbiguanide with powerful antimicrobial activity.<sup>4</sup> Several *in vitro* and *in vivo* studies have proven the efficacy of CHX mouthrinses<sup>5,6</sup> and 0.2% CHX was accepted as the gold standard.<sup>4</sup> Despite great benefits, its side effects, such as extrinsic tooth and tongue staining, enhanced calculus formation, taste aberrations, and rarely painful desquamations of the oral mucosa, are associated with its concentrations and duration of applications and led to the research of new formulations as well as new treatment regimens.<sup>7–11</sup>

Gel form is one of the several different CHX formulations that clinicians are interested in. Among the CHX gel studies, there are only limited number of reports on the influences and clinical importance of 0.2% CHX gel. Lander et al<sup>12</sup> reported that even a single irrigation of 0.2% CHX gel had a marked effect in decreasing the percentage of spirochaetes and motile bacteria at sites with moderate to advanced periodontal disease. Vianna et al<sup>13</sup> showed that 0.2% CHX gel, *in vitro*, eliminated *Porphyromonas endodontalis*, *Porphyromonas gingivalis* (P.g.), and *P. intermedia* in 15 seconds. The CHX gel at 0.2% concentration has also been reported for reducing pain after oral mucosal biopsy<sup>14</sup> and removal of mutans streptococci infection in preschool children.<sup>15</sup>

In this study, we aimed to evaluate the effect of 0.2% CHX containing Cervitec gel on periodontal health by investigating the clinical and microbiological parameters on patients undergoing orthodontic treatment with fixed orthodontic appliances.

## Methods and materials

### Patient selection

Twenty-five patients (mean age: 15.24 years; range: 10–24 years) with at least 20 teeth present, who have been undergoing fixed orthodontic treatment at Gazi University Faculty of Dentistry were randomly assigned to age- and gender-matched two groups, namely, the Cervitec gel group (Ivoclar Vivadent AG, Liechtenstein) ( $n = 13$ ) or the control group ( $n = 12$ ). The patients had different malocclusions, and all have been undergoing fixed orthodontic treatment for at least 6 months. Roth straight wire brackets (0.018 in.  $\times$  0.025 in. slot; GAC International Inc., Bohemia, NY, USA) were placed on both the upper and the lower jaw of all patients. All were at the end of the leveling stage with 0.016  $\times$  0.022 inch or 0.017  $\times$  0.022 inch stainless steel archwires placed on both arches.

Individuals did not reveal any sign of periodontal destruction at the clinic and during a radiographic examination. Patients were excluded if they had received periodontal therapy, antibiotics, or an antimicrobial product in the previous 3 months. All of the patients were systemically healthy and patients with any diagnosed systemic disease and condition that might interfere with the results of our study were also excluded. All of the participants were nonsmokers. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in Tokyo 2004. The study protocol has been reviewed and approved by the Ethical Board of Gazi University School of Medicine, and all participating adult patients and parents of minors were asked to give an informed written consent to participate, after a thorough explanation of the safety and potential efficacy of Cervitec gel, and the probability of receiving Cervitec or placebo gel.

### Study design

This was a double-blinded study, characterizing a randomized placebo-controlled trial. The study protocol is summarized in Fig. 1. During their first visits, before the professional prophylaxis, clinical periodontal parameters of the patients were recorded and subgingival plaque samples were collected. Later, all patients received professional prophylaxis and were given detailed oral hygiene instructions as well as the same type of standard toothpastes (STs; Colgate, Istanbul, Turkey) and toothbrushes (Banat, Istanbul, Turkey). The patients were instructed to brush their teeth two times a day with modified Bass technique. Patients maintained self-performed oral hygiene measures between

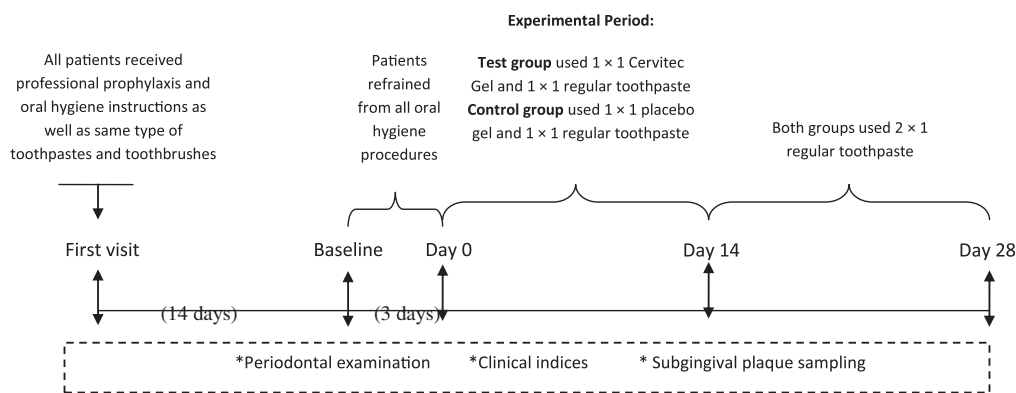


Figure 1 Study protocol.

visits. Two weeks after the first visit, baseline (B) clinical indices and subgingival plaque samples were re-collected. They were asked to refrain from all oral hygiene procedures for 3 days to aid in plaque accumulation. Later, patients were randomly assigned by a single researcher (B.B.T, by toss of a coin) to either the Cervitec group ( $n = 13$ ) or the control group ( $n = 12$ ). The Cervitec group received Cervitec gel, whereas the control group received placebo gel. Patients and other researchers did not know which gel had been delivered to each patient. While the Cervitec group received 0.2% CHX (w/w) and 900 ppm sodium fluoride containing Cervitec gel, the control group received placebo gel, which will be used once a day during tooth brushing, and both the groups were advised to continue brushing once a day with a small pea-sized regular toothbrush. The recommended amount of Cervitec and placebo gel to be used was a full-toothbrush head coverage of the standard toothbrushes provided to all patients. Two weeks later, both groups were asked to brush their teeth two times a day with the previously provided STs. Clinical indices and subgingival plaque samples were repeated at Days 14 and 28.

### Subgingival plaque sample collection and bacterial DNA isolation

In each patient, first maxillary premolars with bracket (right premolar if the patient was left handed) were selected as the representative teeth. Microbiological sampling was performed at B, Day 0, 14, and 28. After removal of supragingival plaque with sterile curettes, the sampling sites of the representative teeth and the adjacent teeth were gently washed, air dried, and isolated with sterile cotton rolls. Sterile paper points (number 40) were inserted for 20 seconds in the gingival crevice and then placed into sterile tubes and stored at  $-20^{\circ}\text{C}$ . Paper points were dipped in bead suspension and agitated by vortexing. The supernatants were cleared from cellular debris and most proteins, and loaded into silica matrix spin columns. After the washing step, DNA was eluted by DNase- and RNase-free water (UltraClean Microbial DNA Isolation Kit; MO BIO Laboratories Inc., Carlsbad, CA, USA).

### Clinical parameters

Full-mouth clinical indices and clinical indices of sampled sites were recorded at the first visit, B, Days 0, 14, and 28

by two previously calibrated examiners (B.Ö. and Ş.B). The clinical examination included modification of the Quigley–Hein Plaque Index (mQHI)<sup>16</sup> with the use of 0.2% erythrosine disclosing agent, gingival index (GI),<sup>17</sup> PD, clinical attachment level (CAL), and bleeding on probing (BOP). The measurements were made at six sites per tooth (mesiovestibular, midvestibular, distovestibular, mesiolingual, midlingual, distolingual) using the Williams periodontal probe calibrated in millimetres (Nordent Manufacturing Inc., Elk Grove Village, IL, USA).

### Quantitation of bacterial load using real-time PCR

Paper-point DNA isolates were used as template DNA in bacterial load quantitation reactions. For each sample, three real-time PCRs were set to assess the total bacterial, P.g., and *Treponema denticola* (T.d.) recombinant DNA (rDNA) copy numbers. In each real-time PCR, the relevant series of quantification standards of P.g. ATCC 33277, T.d. ATCC 35405, and *Escherichia coli* JM109 (*E. coli*) bacterial culture suspensions were run along with the samples, in order to calculate absolute target DNA copy number in the samples. The reaction tubes contained 1 × TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.5  $\mu\text{M}$  of forward and reverse primers, 0.2  $\mu\text{M}$  of fluorescent-tagged 5' hydrolysis probe and 2  $\mu\text{L}$  of sample or standard DNA. The primer and probe sets for total bacterial and specific pathogen rDNA copy number quantification are given in Table 1. Real-time PCRs were carried using fluorometric thermal cycling real-time device (LightCycler 480; Roche Molecular Systems, Alameda, CA, USA). Data analysis was performed using the real-time PCR device software. At the end of each reaction, the CtDNA copy number curve of quantification standards was analyzed and  $R^2$  value was calculated. Absolute DNA copy numbers of samples were calculated using the CtDNA copy number curve of the standards.

### Statistical analysis

Statistical analysis was performed using SPSS version 15.0. Using Shapiro–Wilk normality test, the distribution of data was tested for normality before the analysis. All data were expressed as mean  $\pm$  standard deviation. Test of significance was two tailed and  $P < 0.05$  was considered statistically significant. Friedman test was

**Table 1** Primer and probes used in the study.

<i>Porphyromonas gingivalis</i> rDNA	
Pg16SF	5'-TCGGTAAGTCAGCGGTGAAAC-3'
Pg16SR	5'-ATGGCAAGCTGCCTTCGCAAT-3'
Pg16SP (probe)	5'-FAM-CTCAACGTTACGCTGCCGT TGAAA-TAMRA-3'
<i>Treponema denticola</i> rDNA	
Td16SF	5'-GAATGTGCTCATTTACATAAAGGT-3'
Td16SR	5'-GATACCCATCGTTGCCCTTGGT-3'
Td16SP (probe)	5'-FAM-ATGGGCCCGCGTCCCATTAG CT-TAMRA-3'
Universal bacterial rDNA	
Uni152F	5'-CGCTAGTAATCGTGGATCAGAATG-3'
Uni220R	5'-TGTGACGGGCGGTGTGTA-3'
Uni177T (probe)	5'-FAM-CACGGTGAATACGTTCCCGGG C-TAMRA-3'

rDNA = recombinant DNA.

used to determine differences between groups over time. Time comparisons within groups were tested by Cochran Q test.

## Results

A total of 13 patients who have been undergoing fixed orthodontic treatment (five males and eight females with a mean age  $15.46 \pm 2.85$  years, between 13 and 21 years) and 12 patients for control group (six males and six females with a mean age of  $15.17 \pm 3.30$  years, between 11 and 23 years) were included in this study. All patients completed the study, with no soft tissue exfoliation or interference on taste. The descriptive measurements of clinical indices are summarized in Table 2.

## Clinical findings

The periodontal indices of the sampled sites improved after periodontal prophylaxis and renewal of oral hygiene instructions from first visit to B (Table 2). Significant reductions were detected for mQHI scores of the Cervitec group from first visit to Day 28 ( $P < 0.05$ ), Day 0 to Day 14 ( $P < 0.01$ ), and Day 28 ( $P < 0.001$ ). At the control group, a statistically significant reduction was found from B to Day 0 ( $P < 0.01$ ) and Day 0 to Day 28 ( $P < 0.001$ ). Considering GI scores of the sampled sites, remarkable reductions of GI scores was seen in both the groups; however, only statistically significant alteration was seen in the control group from first visit to Day 28 ( $P < 0.05$ ). Sampled site, PD, CAL, and BOP results revealed no significant alterations in both groups and between the two groups.

## Microbiological findings

The mean values of the total bacteria counts are shown for all examined time intervals in Fig. 2. The mean bacterial counts for P.g. and T.d. are shown in Figs. 3 and 4, respectively. Considering real-time PCR quantitative analysis of the total bacteria, P.g., and T.d., no statistically significant difference was detected between groups or sampling periods in this study.

## Discussion

To our knowledge, this study appears to be the first to evaluate the short-term clinical and microbiological influences of 0.2% CHX and 900 ppm sodium fluoride containing Cervitec gel on patients undergoing fixed orthodontic treatment at least for 6 months.

The present data revealed that from first visit to B, periodontal prophylaxis and oral hygiene instructions

**Table 2** The mean and standard deviations of clinical indices of the sampled sites.

Clinical indices	Groups	First visit	Baseline	Day 0	Day 14	Day 28
mQHI (scores from 0 to 5)	Cervitec	2.69 (0.75) <sup>a,b</sup>	2.12 (0.92)	3.04 (0.86) <sup>c,d</sup>	1.73 (0.86) <sup>c</sup>	1.54 (0.99) <sup>b,d</sup>
	Control	2.46 (1.10)	1.37 (0.68) <sup>e</sup>	2.77 (0.60) <sup>e,f</sup>	1.67 (0.81)	1.42 (0.82) <sup>f</sup>
GI (scores from 0 to 3)	Cervitec	0.63 (0.62)	0.35 (0.38)	0.44 (0.37)	0.37 (0.35)	0.29 (0.32)
	Control	0.90 (0.56) <sup>g</sup>	0.48 (0.52)	0.63 (0.46)	0.29 (0.37)	0.21 (0.30) <sup>g</sup>
PD (mm)	Cervitec	2.11 (0.47)	2.06 (0.55)	2.26 (0.49)	2.02 (0.51)	1.97 (0.82)
	Control	2.45 (1.53)	2.00 (0.65)	2.01 (0.51)	2.19 (0.67)	2.22 (0.57)
CAL (mm)	Cervitec	2.11 (0.47)	2.06 (0.55)	2.24 (0.46)	2.22 (0.50)	1.97 (0.82)
	Control	2.45 (1.53)	2.00 (0.65)	2.01 (0.51)	2.19 (0.67)	2.22 (0.57)
BOP (%)	Cervitec	7.69 (21.37)	2.08 (7.22)	0.00 (0.00)	1.92 (6.93)	1.92 (6.93)
	Control	16.67 (28.87)	2.27 (7.54)	4.67 (9.65)	4.17 (9.73)	0.00 (0.00)

BOP = bleeding on probing; CAL = clinical attachment level; GI = gingival index; mQHI = Quigley–Hein Plaque Index; PD = pocket depth.

<sup>a</sup> Normally distributed data are expressed as means and standard deviations. Friedman test was used for comparisons.

<sup>b</sup> For mQHI, the differences were significant between first visit and Day 28 ( $P < 0.05$ ).

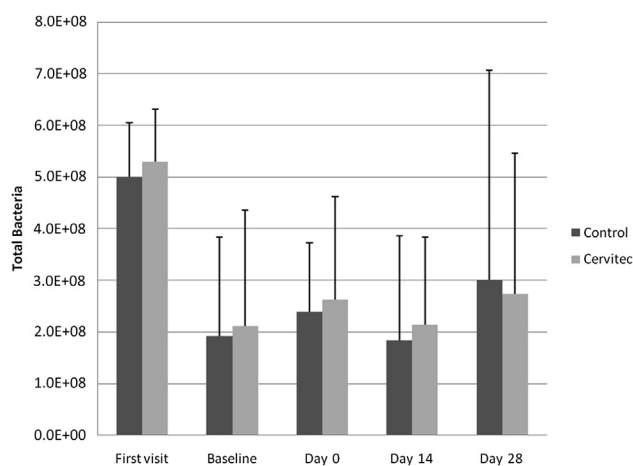
<sup>c</sup> For mQHI, the differences were significant between Day 0 and Day 14 ( $P < 0.01$ ).

<sup>d</sup> For mQHI, the differences were significant between Day 0 and Day 28 ( $P < 0.001$ ) in the Cervitec group.

<sup>e</sup> The differences were significant between baseline and Day 0 ( $P < 0.01$ ).

<sup>f</sup> The differences were significant between Day 0 and Day 28 ( $P < 0.01$ ) in the control group.

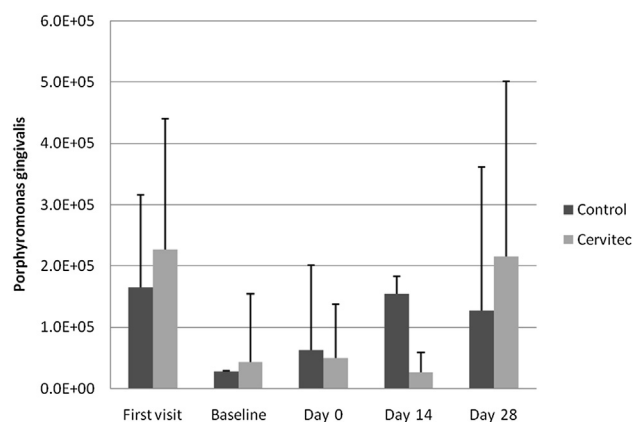
<sup>g</sup> For GI, the differences were significant between first visit and Day 28 ( $P < 0.05$ ) in the control group.



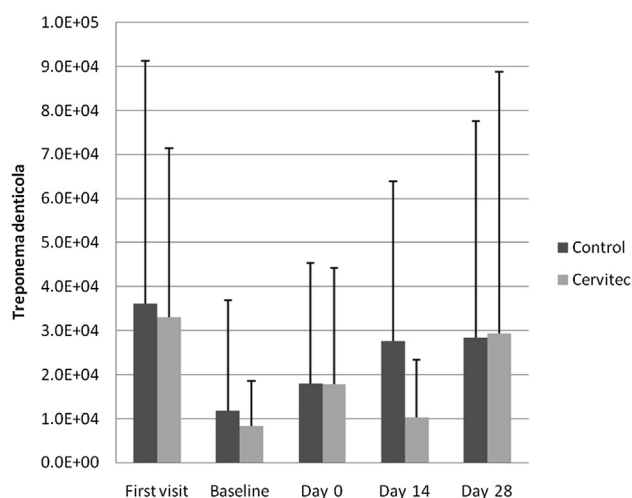
**Figure 2** Comparison of the Cervitec and control groups' real-time PCR quantification of total bacteria in subgingival plaque. The data are expressed as mean and standard deviation.

improved clinical periodontal indices and remarkably reduced total bacteria, P.g., and T.d., although the differences were not always statistically significant. This result confirms the importance of repeated periodontal treatment and oral hygiene instructions and motivation caused by the frequent clinician–patient contact.

When 2 weeks of once-a-day brushing with Cervitec was compared with placebo, clinical indices revealed that plaque accumulation reduced significantly only in the Cervitec group during the experimental period from Day 0 to Day 14 ( $P < 0.01$ ). Nevertheless in the control group, reduction was significant only at Day 28 ( $P < 0.01$ ). In contrast to our findings, Soukoulis and Hirsch<sup>18</sup> reported that after 4 weeks of brushing with 0.2% CHX gel two times a day, there were better plaque score changes in the CHX group compared with the placebo group, but the difference was not significant. They also reported reductions in GI and papillary bleeding index after 4 weeks, with only the GI reductions being significant.<sup>18</sup> In contrast to our study, Soukoulis and



**Figure 3** Comparison of the Cervitec and control groups' real-time PCR quantification of *Porphyromonas gingivalis* in subgingival plaque. The data are expressed as mean and standard deviation.



**Figure 4** Comparison of the Cervitec and control groups' real-time PCR quantification of *Treponema denticola* in subgingival plaque. The data are expressed as mean and standard deviation.

Hirsch<sup>18</sup> recruited patients between 18 and 60 years of age with moderate to severe gingivitis, and poor compliance in gel usage was reported among patients.

Total bacteria, P.g., and T.d. quantitatively increased as expected during 3 days of plaque accumulation but no significant difference was found between groups. Although both P.g. and T.d. increased from Days 0 to Day 14 in the control group, reductions were noteworthy at the Cervitec group during the same period. Moreover, when the B and Day 14 data are considered, it can be suggested that daily use of Cervitec helps to downregulate P.g. and T.d. colonization at the subgingival microbial dental plaque. Although the effect of CHX against the subgingival spirochetes has been reported to be only moderate or weak and often of short duration,<sup>19</sup> our data supported the previous reports<sup>20,21</sup> about important effects of CHX on T.d. and P.g. The CHX kills microorganisms by compromising the integrity of the cell membrane and reported to require a shorter exposure time than, for example, a bacteriostatic agent, such as tetracycline, which inhibits protein synthesis.<sup>22</sup> Our present data are also in agreement with some previous studies that reported the effectiveness of CHX in controlling plaque in different groups and by different ways of application.<sup>13,23–26</sup>

After cessation of Cervitec and placebo gel use (from Day 14 to Day 28), both bacterial counts were increased in the Cervitec group, while P.g. reduced and T.d. continued to increase with a milder pattern in the control group. The number of total bacteria increased in both groups. However, none of those changes were statistically significant. Previously, it was stated that CHX had an affinity for hydroxyapatite, tooth surfaces, and salivary mucins, and that the adsorbed CHX is released slowly when the concentration in the environment is low.<sup>27</sup> It was also suggested that when it is applied by tooth brushing, it can be carried to the binding sites around the teeth and oral cavity, from where a slow release occurs.<sup>27,28</sup> Within the limits of our study, even if the slow release of CHX occurred after cessation of daily brushing with Cervitec gel, our data failed to present some

noteworthy clinical or microbiological effects of CHX after cessation. Present results revealed that there were no differences in the means of P.g. and T.d. between two groups after cessation of daily Cervitec gel use. However, it should also be considered that examining the effects of CHX gel depends on numerous variables, such as different concentrations, formulations, administration time, and forms.<sup>29,30</sup> Because of these constraints, it is hard to make direct comparisons among studies. Previously, it was stated that the effects of CHX gel also depend on the skill of patients to deliver the gel to the appropriate areas of the mouth.<sup>28</sup> Saxen et al<sup>29</sup> suggested that CHX gel does not easily penetrate to the areas other than its site of application.

There are only limited number of studies on the influences and clinical importance of 0.2% CHX gel. Vianna et al<sup>13</sup> evaluated *in vitro* antimicrobial activity of 0.2%, 1%, and 2% CHX in gel and liquid formulations against several planktonic bacteria including P.g., and reported that all tested formulations were able to eliminate P.g. after 15 seconds. By contrast, in our study, patients used 0.2% CHX containing Cervitec gel once a day during brushing for 2 minutes, but no statistically significant alteration at P.g. and T.d. levels within subgingival plaque could be detected. This disparity between two results may be due to the different natures of sessile cells of biofilm and planktonic cells. As different from Vianna et al, in our study we *in vivo* examined clinical and microbial effects of CHX against P.g. and T.d. within subgingival plaque biofilm. It is well described before that each biofilm bacterium lives in a customized microniche in a complex microbial community so that it differs fundamentally from a planktonic cell of the same species.<sup>31,32</sup> Previously, it was reported that planktonic bacteria are more susceptible to the effects of antibiotics and to environmental and host factors, whereas sessile bacteria are able to resist or evade such destructive factors by forming aggregates, altering their physiology, and taking advantage of deficiencies in the host clearance mechanisms.<sup>31–33</sup>

In the present study, we evaluated quantitative changes within subgingival microflora by daily Cervitec gel use, but we did not examine whether their pathogenic properties are affected. Ostela et al<sup>34</sup> examined the effects of CHX–sodium fluoride gel on salivary mutans streptococci and reported that the number of bacteria reduced after gel application and adherence of mutans streptococci was also reduced. In future studies, not only quantitative and qualitative alterations in dental plaque, but also the effects of Cervitec on pathogenic properties of specific periodontal bacteria should be addressed.

In this study, GI was used to evaluate clinical gingival inflammation. Although some significant reductions were detected in each group between observation periods, no significant alterations were detected between the Cervitec and control groups. To obtain more information about alterations of the gingival inflammation, in future studies, inflammatory and proinflammatory cytokine levels within gingival crevicular fluid should be also examined.

To sum up, in this study, both oral hygiene practices ended up with successful clinical results, and the present data suggested that daily use of Cervitec gel appears to have a considerable potential at reducing dental plaque accumulation around teeth and maintaining good oral

hygiene in patients wearing fixed orthodontic appliances. Moreover, the clinical and the bacteriological findings of this study seem to be in line. Even though no statistical significance could be detected between groups or time intervals for quantitative bacterial alterations, P.g. and T.d. reductions seen at the experimental period within subgingival dental plaque were notable. Further studies conducted with larger adult groups, possibly with the history of periodontal disease and longer research periods will clarify the potential of daily Cervitec use within oral hygiene measures.

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