Effect of a self-etching adhesive containing an antibacterial monomer on clinical periodontal parameters and subgingival microbiologic composition in orthodontic patients

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Introduction: The aims of this study were to evaluate the effect of a self-etching adhesive system containing an antibacterial monomer on periodontal health and subgingival microbiologic composition in orthodontic patients and to compare it with a conventional adhesive system. Methods: A split-mouth design was chosen, and 15 patients were included in the study. Brackets in contralateral quadrants were bonded with either a conventional adhesive system (control) or a self-etching adhesive system that contained an antibacterial monomer. Clinical periodontal parameters including plaque index, gingival index, probing depths, and bleeding on probing were determined. Subgingival plaque samples were collected before bracket placement (T0) and at the 6-month follow-up (T1). The real-time TaqMan polymerase chain reaction assay was used to determine the subgingival counts of Porphyromonas gingivalis, Tannerella forsythensis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Campylobacter rectus. For clinical periodontal parameters, analysis of covariance (ANCOVA) and, for bacterial counts, Wilcoxon tests were used for statistical comparisons at the P < 0.05 level. Results: Clinical periodontal parameters were not changed, and they were not different between the groups from T0 to T1. T forsythensis and F nucleatum increased during the treatment period in both groups (P < 0.05). The majority of the bacteria were T nucleatum at T0 and T1 in both groups. Changes in bacterial load from T0 to T1 were not different between groups except for T forsythensis and F nucleatum (P < 0.05). Conclusions: The use of an antibacterial monomer did not have an additional positive effect on clinical periodontal parameters. When used in bonding orthodontic brackets, the antibacterial monomer failed to reduce periodontopathogenic bacteria when compared with the conventional adhesive system during a 6-month treatment period. (Am J Orthod Dentofacial Orthop 2011;140:e147-e153)
are periodontopathic microorganisms predominantly found in subjects with periodontal disease.  

In the attempt to control bacterial plaque around the fixed appliances, a constant level of fluoride was tried to be maintained in the oral cavity followed by oral hygiene instructions and dietary control. Also, combined use of antimicrobials and agents containing fluoride has been suggested for plaque control.

A self-etching adhesive system containing an antibacterial monomer, 1,2-methacyloxy dodecylpyridinium bromide, has antibacterial activity against oral streptococci, and this monomer can be active even after being immobilized as 1 component of a cured composite. Based on the results obtained for this material, a new single-bottle primer containing 5% of this antibacterial monomer was developed, and this 2-step mild self-etching and fluoride-releasing adhesive system with this primer was marketed.

The bonding ability of the self-etching adhesive system containing an antibacterial monomer has been evaluated in vivo, and its antibacterial effects have been demonstrated by in-vitro studies. Although its antibacterial effects against cariogenic bacteria are well documented, no study has investigated the efficacy of this material on periodontopathic microbial composition around the teeth with orthodontic brackets.

The aims of this study were to evaluate the effect of a self-etching adhesive system containing an antibacterial monomer on periodontal health and subgingival microbiologic composition around the teeth with orthodontic brackets.

The null hypothesis of this study was that there were no statistically significant differences among periodontal indexes and subgingival microbiologic composition of teeth bonded with either the self-etching adhesive system containing an antibacterial monomer or the conventional adhesive system.

**MATERIAL AND METHODS**

This study was approved by the ethical committee on research of the Gulhane Military Medical Academy in Ankara, Turkey.

Fifteen randomly selected orthodontic patients (7 boys, 8 girls; mean age, 14.40 ± 0.71 years) participated in the study. All participants required fixed orthodontic treatment for their misaligned teeth. They were in good health; none had taken antibiotics for 3 months before the baseline or during treatment, and none had clinical signs of gingival inflammation. Patients who had undergone periodontal treatment within 3 months before the baseline and those with a systemic disorder that could influence periodontal and microbiologic conditions or the response to treatment were excluded. All subjects provided written informed consent.

This study was organized with a split-mouth design with contralateral antagonistic quadrants receiving the experimental material and the control. Stainless steel orthodontic brackets (3M Unitek, Monrovia, Calif) were bonded by the same operator (M.A.) in the first quadrant, and the second quadrant was the contralateral side. In the control group, teeth in contralateral quadrants were etched for 15 seconds with 37% ortho-phosphoric acid (3M Dental Products, St Paul, Minn), rinsed with water from a 3-in-1 syringe for 15 seconds, and dried with an oil-free source for 15 seconds. Before bracket placement, Transbond XT primer (3M Unitek) was applied to the etched surface in a thin, uniform coat. The primer was cured for 10 seconds with a light-emitting diode unit (Ortholux XT, 3M Unitek). Adhesive paste (Transbond XT, 3M Unitek) was applied to the bracket base, and the bracket was positioned on the facial surface and pressed firmly into place. The excess adhesive around the bracket was carefully removed with a scaler.

In the experimental group, teeth in the remaining quadrants were etched similarly to the control group for 15 seconds. First, Clearfil Protect Bond self-etch primer (Kuraray Medical, Okayama, Japan) was applied to the etched surface for 20 seconds and sprayed with a mild airstream to evaporate the solvent. Then Clearfil Protect Bond was applied, gently air dried, and light cured for 10 seconds. Finally, a thin layer of Transbond XT adhesive paste was applied to the base of the bracket and immediately pressed into the adhesive on the tooth surface.

Seven days before the baseline examinations, the subjects had oral hygiene instructions. During the treatment, the subjects did not receive prophylaxis procedures. A full periodontal examination, including plaque index, gingival index, probing depth, and bleeding on probing, was performed before bonding (T0) and 6 months after the beginning of orthodontic treatment (T1). Probing depth and bleeding on probing were obtained at 6 sites per tooth; the plaque and the gingival indexes were determined for the labial and lingual sites separately. Periodontal evaluation was carried out by the same trained clinician (S.E.) in all patients using a marked periodontal probe (Hu-Friedy, Chicago, Ill). This clinician was blinded to the group allocations. Probing depth was measured to the nearest millimeter on the scale.

In every recording, microbiologic samples were collected before the clinical procedures by the same clinician (S.E.). Subgingival plaque samples were collected...
from the mesiobuccal gingival crevices of the second premolars and the lateral incisors in each quadrant (maxillary right second premolars and lateral incisors, and mandibular left second premolars and lateral incisors for 1 group, and teeth in the contralateral quadrants for the other group).

Sampling sites were isolated with sterile cotton rolls and dried by a gentle airstream. Then a sterile curette was gently inserted to the bottom of the test periodontal pocket, and subgingival material was removed with a single stroke; pooled samples were stored in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) at −80°C that contained 400 µL of distilled water.

Before DNA extraction, the deep-frozen samples were thawed and dispersed by vortexing for 15 seconds. DNA was extracted from the clinical sample material by using an alkali phenol-chloroform-isoamyl alcohol procedure. Briefly, 120 µL of a specimen was placed in 12 µL of proteinase K solution (20 mg/mL) (Sigma-Aldrich, St Louis, Mo) and 500 µL of potassium buffer for 60 minutes at 55°C. After centrifugation at 10,000 × g for 10 minutes at 12°C, DNA was extracted from the supernatant by using a mixture of 250 µL of alkali phenol and 250 µL of chloroform-isoamyl alcohol, and then precipitated by using 500 µL of isopropyl alcohol. DNA was washed in 75% ethyl alcohol at 10.000g for 5 minutes at 4°C, air dried at 37°C, and dissolved in 200 µL of distilled water.

The TaqMan fluorogenic real-time polymerase chain reaction detection system was used to determine infectious agent counts. The TaqMan system uses species-specific primers and probes that are dually labeled with a fluorescent reporter and a quencher dye. The Taq probe cleaves the TaqMan probe during the polymerase chain reaction amplification process, separating the reporter from the quencher dye, which increases the intensity of the reporter fluorescence proportionally to the starting copy counts of the target DNA. Table I lists the nucleotide sequence of the polymerase chain reaction primers and probes (Oligoware version 3.0, Medical Microbiology Department, Gulhane Military Medical Academy, Ankara, Turkey). The primers and probes were synthesized by Metabion International (Pla-negg-Martinsried, Germany). Infectious agents included in the study were P gingivalis, T forsythensis, P intermedia, A actinomycetemcomitans, F nucleatum, and C rectus. For each infectious agent studied, polymerase chain reaction amplification was performed as an individual assay. Amplification, data acquisition, and all analyses were carried out by using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, USA).

### Table I. Polymerase chain reaction primers and TaqMan probes for detecting bacteria

<table>
<thead>
<tr>
<th>Infectious agents (GenBank accession number)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphymonas gingivalis (AB261608.1)</td>
<td>194</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-TGGGACTTTGCTTCTTTGCTATG-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GATGGCTTCCTCTGCTTTCCCA-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>FAM-5'-CAAGACAAACAGAGCGAAGCCCTTA-TAMRA-3'</td>
</tr>
<tr>
<td>Tanneryella fornythia (DQ344918.1)</td>
<td>149</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-GGTATGTAACCTGCCCCCA-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-CGTTACCTCACCAGTACTAAGT-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>FAM-5'-AGGGATAACCCGGGAAAGCTCGGA-TAMRA-3'</td>
</tr>
<tr>
<td>Prevotella intermedia (AY689226.1)</td>
<td>105</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-AGACGGCCCTAATACCGGATGTG-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-TTACCGCACCACAAAAGCTAATCG-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>JOE-5'-TGCGATCTGACGGACAAAGATTC-TAMRA-3'</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans (AF35951.1)</td>
<td>288</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-CGTTACGTTATGACCGTGTA-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GCGCGGAATGCTTTGCTATATTTTC-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>FAM-5'-AGGGAAACCGGGAAGCTAACGAAA-TAMRA-3'</td>
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<tr>
<td>Fusobacterium nucleatum (EF089177.1)</td>
<td>175</td>
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<tr>
<td>Forward</td>
<td>5'-GGCAATCACAAGTACAGGTTG-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GTCGACCACCCACACATCAGTA-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>JOE-5'-AAATGGCGATGGGGAAGCCACTCA-TAMRA-3'</td>
</tr>
<tr>
<td>Campylobacter rectus (AF0351193.1)</td>
<td>132</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-CCCGATACACCTTACTCTCTTA-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GATCCGTTACCTCAGATCCACTA-3'</td>
</tr>
<tr>
<td>Probe</td>
<td>FAM-5'-CCGGTACCAATCTCCTGAGGAAACATA-MARMA-3'</td>
</tr>
</tbody>
</table>

FAM, 6-carboxyfluorescein labeled reporter dye; JOE, 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein labeled reporter dye; TAMRA, 6-carboxytetramethyl-rhodamine labeled quencher dye; bp, base pairs.
Foster City, Calif). For each infectious agent tested, the TaqMan polymerase chain reaction assay was performed with a final volume of 25 μL of reaction mixture, containing 5 μL of extracted clinical sample, 12.5 μL of TaqMan universal polymerase chain reaction master mix (Applied Biosystems), 5 pmol of primers, and 3 or 4 pmol of TaqMan probe. The polymerase chain reaction cycling program included 2 minutes at 50°C to eliminate carryover contamination, 10 minutes at 95°C to activate the hot-start Taq DNA polymerase (AmpliTaq Gold DNA Polymerase, Applied Biosystems), and then 40 cycles, with each cycle consisting of 2 steps at 60°C for 1 minute and 1 step at 95°C for 15 seconds. Bacterial copy numbers were multiplied by 100 to adjust for sample dilution in the polymerase chain reaction assay. Polymerase chain reaction quantification standards included plasmids containing bacterial amplicons, which were cloned by using a cloning kit (TOPO-TA; Invitrogen, Carlsbad, Calif). The 6 tested bacteria showed no cross-reactivity with even closely related species, such as prevotellanigrescens. Also, a blast search (National Center for Biotechnology Information, Bethesda, Md), to check the specificity of the primers and probes used, showed no genomic cross-reactivity with other mammalian viruses, bacteria, or cells. The dynamic range of quantification of the TaqMan polymerase chain reaction assay was determined by serial dilution of the plasmid-generated standards in the range of 109 to 101 copies per milliliter.

**Statistical analysis**

All statistical analyses were performed by using the Statistical Package for the Social Sciences (version 15.0; SPSS, Chicago, Ill). The Shapiro-Wilks normality test and the Levene variance homogeneity test were applied to the data. Clinical periodontal parameters showed normal distribution; there was homogeneity of variances between the groups. The effects of the different adhesive systems on the groups were evaluated with analysis of covariance (ANCOVA).

Counts of bacteria were recorded by conversion to logarithmic values, but they did not show normal distribution; there was no homogeneity of variances between groups. Intragroup and intergroup comparisons were evaluated by using the nonparametric Wilcoxon test. The statistical significance level was set at \( P < 0.05 \).

**RESULTS**

Because of the oral hygiene instructions given 1 week before the placement of fixed appliances, the patients were good in periodontal health. In the experimental and the control groups, after the bonding of brackets,
the results of ANCOVA analyses showed that the different adhesive systems did not have significant effects on the groups during the study ($P > 0.05$) (Table II).

Descriptive values and statistical comparisons of bacterial loads in the plaque samples are given in Table III. Comparisons at T0 were not significant between the groups. Changes in bacterial composition from T0 to T1 were not different for both groups except for *T. forsythensis* and *F. nucleatum* ($P < 0.05$). *F. nucleatum* was the most frequently found bacteria at T0 and T1 in both groups. The amounts of *T. forsythensis* ($P < 0.05$) and *F. nucleatum* ($P < 0.05$) changed, but *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *C. rectus* remained unchanged during the treatment period.

**DISCUSSION**

In the literature, there are many studies investigating new materials in conservative and restorative dentistry. As new materials and techniques are introduced, orthodontists adopt some innovations and add them to their routine armamentarium, including self-etching primers, resin-modified glass ionomers, varnishes containing chlorhexidine or fluoride, flowable composites, amorphous calcium phosphate-containing composites, and various adhesives.17

Antibacterial agents could be integrated into adhesive systems. A self-etching adhesive system with an antibacterial primer is also claimed to release fluoride. When composite containing a self-etching adhesive with an antibacterial monomer is polymerized, the bonding interface is considered to be maintained for a long time.18 Furthermore, cured primer incorporating the antibacterial monomer inhibits bacterial growth on its surface by immobilized antibacterial components.11

For ethical considerations and to limit the sample size, the effects of various products can be investigated by using a split-mouth study design. This method was chosen to reduce individual variations and intragroup differences; however, there are some deficiencies and difficulties related to the possibility of bacterial contamination and the carry-across effect from the antibacterial monomer.6

The evaluation of periodontal parameters might have the risk of interobserver differences. Therefore, all patients were examined by the same clinician. All periodontal indexes used in this study were recorded to determine the health of ginvial tissues and alterations in the periodontium. At the beginning of the study, the results showed minimum values that indicated healthy periodontiums for all indexes just before the start of orthodontic therapy. This was achieved by the positive effects of oral hygiene instructions given a week before placement of the fixed appliances. Our patients were told to maintain their normal oral hygiene regimen to imitate more closely the real situation of plaque development during fixed appliance treatment.

The placement of fixed appliances caused increased plaque accumulation, pocket depth, and gingival inflammation in the short term. Thus, the host-microorganism balance is changed, and gingival inflammation results in bleeding on probing. The clinical periodontal indexes remained relatively constant over the observation period in both groups. These results confirm the lack of periodontal destruction in patients with fixed appliances and failed to demonstrate significant increases in the clinical periodontal indexes in short time periods.19,20 It could be concluded that all reactions during fixed orthodontic therapy are the consequences of ginvial reactions and not the result of deterioration of deeper periodontal tissues.

After enamel decalciﬁcation and cariogenic bacteria were studied widely in the orthodontic literature,21 periodontopathogenic microorganisms have become a popular topic of interest.22 The microflora colonizing the oral cavities consist of numerous bacterial species; most are innocuous, but colonization of the subgingival plaque by certain species can lead to periodontal disease.23

In the detection of anaerobic bacteria, the polymerase chain reaction method is much more sensitive and has greater specificity compared with other microbiologic identiﬁcation techniques such as cell culturing and the DNA probe method.24 Real-time polymerase chain reaction, also called quantitative real-time polymerase chain reaction, is an laboratory technique based on polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute numbers of copies or relative amounts when normalized to DNA input or additional normalizing genes) of at least 1 speciﬁc sequence in a DNA sample. In this study, the detection of putative periodontal pathogens from the subgingival plaque samples was performed by real-time polymerase chain reaction because of its high sensitivity and rapid detection and quantification of individual microbial species. But only a few studies have used polymerase chain reaction methods to evaluate microbial alterations in orthodontic patients.25

Gram positive and aerobic microorganisms are the first colonizers of intraoral hard surfaces then they are replaced by gram negative and anaerobic microorganisms that are known to have harmful effects on the periodontium.23 *T. forsythensis*, *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia* were found more frequently in patients with gingivitis and periodontitis than in healthy subjects.26 Elevated levels of *P
gingivalis, P. intermedia, T. forsythensis, and fusobacterium species were found in the experimental group after bracket placement compared with the control group without orthodontic therapy. T. forsythensis, T. denticola, and P. nigrescens were significantly more common in the samples obtained from orthodontic patients than in the samples obtained from the nonorthodontic control patients. Associated with gingivitis, C. rectus was also investigated.

Considering the presence of periodontopathogens, our study agreed with a previous report that demonstrated that putative periodontal pathogen colonization is established in the early years of life. In plaque samples, F. nucleatum was the most frequently found pathogen and was increased significantly in the experimental and conventional adhesive groups. F. nucleatum can aggregate with many bacterial species and bind to host tissues and immunoglobulin A, allowing F. nucleatum to invade epithelial cells and participate in plaque formation. This microorganism also can be found in periodontal health and inflammation and could represent a triggering or supporting factor for disease progression. A high prevalence of this microorganism supports the hypothesis mentioned above.

The second most prevalent microorganism, T. forsythensis, increased during the treatment period, but it also occurs in healthy sites. Contrary to the findings of Gafan et al., a significant bacterial shift was observed for T. forsythensis in our study. Therefore, we suggest that this microorganism might be an initiative factor for disease progression. The 2 most prevalent microorganisms also increased during treatment in both groups, and they are considered to be potential mucosal invaders. The presence and progression of these 2 prevalent microorganisms can be considered a natural occurrence in this age cohort.

Only some specific forms of subgingival bacteria have direct detrimental effects on periodontal tissues. The most dangerous microorganisms that can cause periodontal disease are anaerobes such as P. intermedia, A. actinomycetemcomitans, P. gingivalis, and others. Zadeh et al.13 suggested that destruction of the periodontium by A. actinomycetemcomitans is caused by the interaction between this pathogen and the host’s response. In a previous report, the authors found a significant increase in the positive findings of A. actinomycetemcomitans in dental plaque samples after placement of fixed appliances. A. actinomycetemcomitans, P. gingivalis, and P. intermedia can penetrate into the buccal epithelial cells in the oral cavity. The presence of these bacteria might be considered a risk factor for the development of disease; therefore, they are part of many tests. A positive test implies a higher risk for the development of the aggressive form of the disease. No significant bacterial shifts were observed for A. actinomycetemcomitans, P. gingivalis, and P. intermedia during treatment for both groups. These results are similar to most previous reports. No deterioration in deeper periodontal tissues and limited gingival inflammation could explain this result. Contrary to the findings by Lee et al., C. rectus had a slight shift during the study.

The use of a self-etching adhesive system containing an antibacterial monomer is considered to improve periodontal conditions because of its antibacterial properties to inhibit inflammation. Periodontal indexes remained unchanged in both the experimental group and the conventionally bonded group. So, the first part of the null hypothesis that there were no statistically significant differences for periodontal indexes between groups cannot be rejected, because at T1 the periodontal indexes were not changed, and there were no significant differences between the groups. The mechanism of the antibacterial activity of the antibacterial monomer is thought to be through contact of the immobilized antibacterial portion on the material surface and the bacteria, causing the bacterial cells to die or become inactive. Most of the periodontopathogens are gram negative, and the anaerobic bacteria antimicrobial effects of the antibacterial monomer might be limited because of different surface structures.

The interesting finding of the study is the significant difference in the amounts of T. forsythensis and F. nucleatum at the end of the study between the groups. This condition can be attributed to inhibition of cariogenic bacteria that might have resulted in periodontopathogenic bacteria. So, the second part of the null hypothesis that there were no statistically significant differences for subgingival microbiologic composition between groups was rejected.

CONCLUSIONS

1. The use of an antibacterial monomer did not have an additional positive effect on clinical periodontal parameters.
2. The antibacterial monomer used for bonding orthodontic brackets failed to reduce the periodontopathic bacteria compared with a conventional adhesive system during a 6-month treatment period.

REFERENCES


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