Effectiveness of a Hydrophilic Primer When Different Antimicrobial Agents Are Mixed

Ali Ihya Karaman, DDS, MS, PhD[a]; Tancan Uysal, DDS[b]

Abstract: The purpose of this study was to determine whether different types of antimicrobial agents with hydrophilic primer applied to etched enamel surfaces will affect the shear bond strength (SBS) and the bracket/adhesive failure modes of metallic orthodontic brackets. Eighty noncarious human premolars were divided into four groups of 20 each. A composite resin (Transbond XT) was used to bond stainless steel brackets. Teeth in the first group were used as a control and bonded with standard procedures. For the other three groups, mixtures containing a hydrophilic primer (Transbond MIP) and one of three antimicrobial agents were prepared (Cervitec®: in 1:2 ratio; chlorhexidine mouthwash and EC40® varnish in 1:1 ratio). These mixtures were applied to the etched enamel surfaces and thoroughly light cured for 20 seconds, and the brackets were bonded and light cured for 40 seconds. The SBS values of these brackets (Mpa) were recorded using a universal testing machine. Adhesive Remnant Index scores were determined after failure of the brackets. Data were analyzed using analysis of variance (ANOVA), Tukey honestly significant difference, and chi-square tests. Results of ANOVA revealed statistically significant differences in bond strengths among the various groups tested ($P < .05$). The bond strength values in these four groups compared favorably with those from other studies and the minimal bond strength values that are clinically acceptable. However, results of this study demonstrated that groups 1 (control) and 2 (Cervitec varnish) had higher SBS values than the other applications. Application of different antimicrobial agents may result in differences in the site of failure. (*Angle Orthod* 2004;74:414–419.)

Key Words: Hydrophilic primer; Chlorhexidine; Antimicrobial agents

INTRODUCTION

The practice of orthodontics is constantly being improved with the use of new techniques and materials that benefit both the patient and the clinician.[1–3] Nevertheless, patients who undergo orthodontic therapy have changes in the oral ecologic, such as a low-pH environment, increased retentive sites for *Streptococcus mutans*, and increased retention of food particles, which may lead to increased proportions and absolute numbers of salivary *S. mutans*.[4–9] These changes may be responsible, in part, for the observations of post–orthodontic treatment decalcification in certain cases.[10,11] Øgaard et al[12] indicated that a high prevalence of carries may be caused by the high cariogenic challenge prevailing in the plaque around orthodontic appliances. Proper oral hygiene is more difficult to maintain, and pH levels lower than 4.5 have been measured in the plaque around the brackets and the bands during orthodontic treatment.[12] At such a low pH, the remineralization phase is hampered and more fluoride will not necessarily give a better cariostatic effect.[13] For that reason, Øgaard and Rølla[13] suggested that fluoride agents could be further improved by the addition of antibacterial agents.

Placement of fixed orthodontic appliances is normally followed by an increase in oral colonization by mutans streptococci, concomitant with an elevated risk for the development of dental caries.[6,14–17] The application of Cervitec varnish induced a significant reduction of *S. mutans* in saliva over a one-month period[18] and a reduction in the proportion of *S. mutans* in the plaque adjacent to brackets. However, no clinical differences were found in the incidence of incipient enamel demineralization around the bracket bases.[19] The differences decreased with time, becoming statistically nonsignificant during the third month. Twetman and Petersson[20] proved an intensive treatment (three applications within a two-week period) was more effective than a monthly application during a three-month period.

Sandham et al[21] treated 26 children with Chlorzoin at

[a] Associate Professor, Selcuk University, School of Dentistry, Department of Orthodontics, Konya, Turkey.

[b] Research Assistant, Selcuk University, School of Dentistry, Department of Orthodontics, Konya, Turkey.

Corresponding author: Tancan Uysal, DDS, Orthodontics, Selcuk Universitesi Dis Hekimligi Fakultesi, Ortodonti A. D., Konya 42079, Turkey
(e-mail: tancanuysal@selcuk.edu.tr).

Accepted: July 2003. Submitted: June 2003.

four time points during the month before initiating orthodontic treatment. Treatment resulted in a decrease in salivary mutans streptococci counts after one week (23 subjects had nondetectable *S. mutans* levels) and one month post-treatment (22 subjects still had nondetectable *S. mutans* levels), gradually decreasing toward the end of the study, with 11 subjects free of detectable *S. mutans* six months later.

Using an agar diffusion inhibitory test,[22] Petersson et al[23] tested the effect of the Cervitec varnish on pure cultures of a series of gram-positive and gram-negative bacteria as well as yeasts. Their results reported *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* as the most sensitive and *Streptococcus sanguis* and *Candida albicans* as the least sensitive. Thymol was considered to be responsible for the antimicrobial effect on *S. sanguis*. Both streptococcal and actinomycetes species were affected by the test varnish, which may be of clinical importance for enamel and root surface caries reduction, as had been suggested previously.[24]

Schaeken and de Haan[25] reported their initial results with a varnish prepared by mixing 50% (wt/wt) chlorhexidine diacetate with a five wt% NaF varnish (Duraphat®, Woelm Pharma, Eschwege, Germany). Gradually, this varnish has been modified and commercialized as EC40® (Certichem, Nijmegen, The Netherlands) and contains 40% chlorhexidine, sandarac, and ethanol.

Bishara et al[1] and Damon et al[2] determined the effects of chlorhexidine varnish on the bond strength of orthodontic adhesives on etched enamel surface. Bishara et al[3] also published the effects of various methods of chlorhexidine application on shear bond strength (SBS). They indicated that SBS is not significantly affected when chlorhexidine is applied if the varnish is premixed with the sealant and applied on the etched enamel surfaces and then light cured. These reports are the starting points of this investigation.

The purpose of this study was to determine whether the application of different types of antimicrobial agents with hydrophilic primer will affect the SBS and the bracket/adhesive failure modes of metallic orthodontic brackets.

MATERIALS AND METHODS

Eighty noncarious human premolars, extracted with orthodontic indications, were used in this study. Teeth with hypoplastic areas, cracks, or gross irregularities of the enamel structure were excluded from the study. The criteria for tooth selection dictated no pretreatment with chemical agent such as alcohol, formalin, hydrogen peroxide etc. The teeth were stored in distilled water continuously after extraction. The water was changed weekly to avoid bacterial growth. The sample was divided into four random groups of 20 each. Each tooth was mounted vertically in self-cure acrylic so that the crown was exposed. The buccal enamel surfaces of the teeth were cleansed and polished with nonfluoridated pumice and rubber prophylactic cups, washed with water, and dried before any procedure.

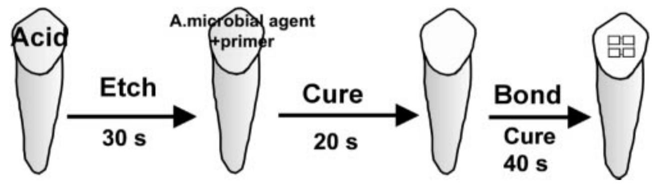

FIGURE 1. Flowchart for the bonding procedure.

Before the starting procedure, the surface of each tooth was polished for one minute using the combination of a polishing agent and a brush at a low speed (3000 rpm). A 37% phosphoric acid gel (3M Dental Products, St Paul, Minn) was used for acid etching of 20 premolars for 30 seconds each. The teeth were rinsed with water for 30 seconds and dried with an oil-free source for 20 seconds. In all etched cases the frosty white appearance of etched enamel was noticed. An orthodontic composite (Transbond XT, 3M Unitek, Monrovia, Calif) was used to bond stainless steel brackets (Dyna-lock series, 3M Unitek). The average surface area for the orthodontic bracket base used was 14.00 mm^2. Excess bonding resin was removed with a scaler. The sealant was light cured for 20 seconds, and the adhesive was light cured for 40 seconds. Except for the control group, all the other groups were prepared according to the recommendations of Bishara et al[3] (Figure 1).

After acid etching, the brackets were bonded in the following manner:

Group 1 (control). The teeth were sealed with primer (Transbond MIP), and then air was blown gently on each tooth for two to five seconds, aiming the air stream perpendicular to the labial surface of the tooth. The brackets were then bonded.

Group 2 (Cervitec® varnish). The chlorhexidine varnish used (Cervitec, Vivadent, Schaan, Lichtenstein) contains equal amounts of chlorhexidine and thymol (1 mg of each/g). Primer and varnish[3] were thoroughly mixed in a 1:2 proportion, applied to the enamel surface, and light cured for 20 seconds, and then the brackets were bonded.

Group 3 (chlorhexidine mouthwash). A mouthwash containing 0.012% chlorhexidine gluconate was used in this group (Drogsan Pharmaceuticals, Ankara, Turkey). Primer and mouthwash were thoroughly mixed in a 1:1 proportion, applied to the enamel surface, and light cured for 20 seconds. The brackets were then bonded.

Group 4 (EC40® varnish). Another chlorhexidine varnish, EC40® (Certichem), containing 40% chlorhexidine, sandarac, and ethanol, was used. Primer and varnish were thoroughly mixed in the same proportion (1:1), applied to the enamel surface, and light cured for 20 seconds, and the brackets were bonded (Figure 1).

Debonding procedure

The embedded specimens were secured in a jig attached to the base plate of a universal testing machine (Micro 500, Testometric, Maywood Instruments Limited, Basingstoke, UK). A chisel-edge plunger was mounted in the movable crosshead of the testing machine and positioned so that the leading edge aimed the enamel-adhesive interface before being brought into contact at a crosshead speed of 0.5 mm/min. The maximum load necessary to debond the bracket was recorded. The force required to take off the brackets was measured in Newtons (N), and the SBS (1 MPa = 1 N/mm^2) was then calculated by dividing the force values by the bracket base area (14.00 mm^2).

Residual adhesive

After debonding, all teeth and brackets were examined at a 10× magnification with light microscopy. Any adhesive remaining after bracket removal was assessed with the adhesive remnant index (ARI)[26,27] and scored with respect to the amount of resin material adhering to the enamel surface. The ARI scale has a range between 5 and 1, with 5 indicating that no composite remained on the enamel; 4 indicating less than 10% of the composite remained on the tooth; 3 indicating more than 10% but less than 90% remained on the tooth; 2 indicating more than 90% of the composite remained; and 1 indicating all the composite remained on the tooth, along with the impression of the bracket base. The ARI scores were used as a more comprehensive means of defining the sites of bond failure between the enamel, the adhesive, and the bracket base.

Statistical methods

Descriptive statistics including the mean, standard deviation, and minimum and maximum values were calculated for each of the four groups of teeth tested. Comparisons of means were made using analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) tests. The chi-square test was used to determine significant differences in the ARI scores among the different groups. All statistical analyses were performed using the SPSS software package (SPSS for Windows, version 10.0.1, SPSS Inc, Chicago, Ill).

RESULTS

Shear bond strength

The descriptive statistics, including the mean, standard deviation, and minimum and maximum values, for each of the four groups are presented in Table 1. Data were analyzed using ANOVA, Tukey HSD, and chi-square tests. The results of this study demonstrated that the group 1 (control) had higher SBS values than the other applications. Results of ANOVA revealed statistically significant differences in bond strengths among the various groups tested ($P < .05$).

TABLE 1. Descriptive Statistics of the Four Groups[a]

Groups Tested[b]	n	Mean	SD	Range	Test[c]
Group 1	20	18.96	4.52	11.1–25.7	A
Group 2	20	16.77	4.26	10.9–26.2	A
Group 3	20	10.60	4.02	5.7–19.7	B
Group 4	20	13.70	2.35	9.2–21.2	B

[a] Values in MPa. n indicates sample size; SD, standard deviation.
[b] Group 1 indicates the control; group 2, Cervitec varnish; group 3, chlorhexidine mouthwash; group 4, EC40 vanish.
[c] Groups with different letters are significantly different from each other.

TABLE 2. Frequencies of ARI Scores[a,b]

Groups Tested	n	1	2	3	4	5
Group 1	20	—	3	6	9	2
Group 2	20	2	3	8	6	1
Group 3	20	—	—	—	2	18
Group 4	20	—	—	—	1	19
$\chi^2 = 77.743$, $P = .0001$						

[a] ARI indicates adhesive remnant index; n, sample size.
[b] ARI scores: 1 indicates all of the composite, with an impression of the bracket base, remained on the tooth; 2, more than 90% of the composite remained; 3, more than 10% but less than 90% of the composite remained on the tooth; 4, less than 10% of composite remained on the tooth surface; 5, no composite remained on the enamel.
[c] Group 1 indicates the control; group 2, Cervitec varnish; group 3, chlorhexidine mouthwash; group 4, EC40 vanish.

The Tukey multiple range analysis indicated that the highest SBS values occurred when the brackets bonded with direct Transbond MIP primer (group 1) (mean 18.96 ± 4.52 MPa) and when the chlorhexidine varnish (Cervitec) was premixed with the sealant and applied to the etched enamel surface (group 2) (mean 16.77 ± 4.26 MPa). On the other hand, when the chlorhexidine mouthwash and the other forms of chlorhexidine varnish (EC40) were premixed with the sealant and applied to the etched enamel surface, significantly lower SBS values were observed, (SBS values of groups 3 and 4, 10.60 ± 4.02 and 13.70 ± 2.35 MPa, respectively).

Adhesive remnant index

The residual adhesive on the enamel surfaces as evaluated by the ARI scores are presented in Table 2. There were statistically significant differences present among the various groups. ($\chi^2 = 77.743$, $P = .0001$). In groups 1 and 2, there was a higher frequency of ARI scores of 3 and 4, which indicated cohesive failures within the resin. Especially in groups 3 and 4, there was no or little adhesive remaining on the tooth (ARI scores 4 and 5); ie, almost all the adhesive stayed on the bracket base. These failures were mostly adhesive at the resin/enamel interface.

DISCUSSION

Hahn et al[28] indicated that microbes accumulate on restorative materials. Among these, mutans streptococci are known to cause secondary caries at the margins of composite restorations as well as directly attack the enamel.[29,30] Chlorhexidine is one of the most widely used broad spectrum antibacterial or antiseptic agents in dentistry.[31] It has proven to be very effective in the maintenance of plaque control and gingivitis in both short-[32] and long-term studies[33] without developing resistant organisms in the oral flora. The prevalence of bacteremia found with chlorhexidine application was less than the prevalences obtained without chlorhexidine.[34] There is evidence in the literature that antiseptic mouthwashes applied before dental manipulations may reduce the incidence and severity of bacteremia.[34] Some of the side effects of using chlorhexidine that limit its widespread acceptance include brown staining of the teeth, an increase in calculus deposition, and the difficulty in completely masking its taste when used as a rinse.[31]

Cervitec, EC40, and other forms of antimicrobial varnishes cannot be found in every clinic, and they are very expensive. However, some agents used as mouthwashes are generally found in dental clinics for irrigation or antiseptic purposes. We believed that for antimicrobial purposes other agents could be used instead of chlorhexidine varnishes before or during orthodontic bonding procedures. Therefore, this project was devised for test the SBS values of different antimicrobial agents. Little or no information is available on the use of liquid forms of antimicrobial agents after etching the enamel and before placing the bracket. Applying chlorhexidine to the enamel surface could add increased protection around the bracket periphery but could also adversely influence the bond strength, depending on the method of application.[3]

In the present study, Tukey HSD analysis revealed that the SBS was not significantly affected after treating the enamel surface with a hydrophilic primer (Transbond MIP) and Cervitec varnish (Table 1). Bishara et al[1] and Damon et al[2] reported that the bond strength was not affected after the application of a hydrophobic primer (Transbond XT) with chlorhexidine varnish on etched enamel. These findings are in accordance with other findings.[1,2] However, the SBS values were significantly affected after treating the enamel surface with the same primer and other forms of antimicrobial varnish EC40. Statistically significant lower SBS values were obtained with these mixtures. Therefore, it was thought that different chemical properties or compositions of varnishes have some effects on SBS values of orthodontic brackets.

Bishara et al[3] indicated that 40% of the brackets tested did not register any debonding force, and the brackets separated with all the adhesive remaining on the bracket base, with a sticky varnish layer almost acting as a separating medium from the enamel surface. In our study, during the preparation of the test materials the SBS value was significantly reduced when liquid forms of antibacterial agents were premixed with the primer and applied to the etched enamel and light cured before applying the adhesive. Despite this reduction, all the brackets tested in the current study registered debonding forces contrary to the results of Bishara et al.[3] We thought that these changes occurred because of using the hydrophilic primer.

Reynolds[35] determined that the clinically acceptable minimal bond strength values in direct orthodontic bonding systems are 5.9 MPa to 7.8 MPa. The bond strength values in all four groups compared favorably with Reynolds'[35] minimal bond strength values. However, clinical conditions may significantly differ from an in vitro setting. It needs to be emphasized that this is an in vitro study and the test conditions have not been subjected to the rigors of the oral environment.[3] Heat and humidity conditions of the oral cavity are highly variable. Because of the probable differences in in vivo and in vitro conditions, a direct comparison cannot be made with the findings of the other studies.

The differences in the SBS values among the four different groups are reflected in the distribution of the ARI scores in Table 2. In groups 3 and 4, the failure rate increased and the site of bond failure shifted statistically significantly toward the composite-varnish interface (ARI scores of 4 and 5). These findings reveal that the adhesive did not effectively bond to the enamel surface as in groups 1 and 2. These findings were similar to Bishara's[3] findings.

Thorough plaque and inflammation control is very difficult in patients with fixed orthodontic appliances; chemical agents such as chlorhexidine or benzydamine, used in the form of mouth rinses or oral sprays, have been shown to be useful adjuncts in plaque and inflammation control.[36] Varnish forms of the other antibacterial solutions such as benzydamine, triclosan, and xylitol could be helpful in orthodontic patients for suppressing levels of oral mutans or the other microbes for long periods after application when used before the placement of fixed orthodontic appliances. For that reason we expect that varnish forms of the other antimicrobial agents will be developed by manufacturers.

The literature indicates that subgingival irrigation with a solution of chlorhexidine, either by single or repeated applications over varying time periods, effectively reduces inflammation and plaque in periodontal patients not undergoing active orthodontic treatment.[37–43] Therefore, further studies investigating the effects of different antimicrobial agents on gingival tissues in the form of subgingival irrigation are needed.

CONCLUSIONS

The findings derived from the study are as follows:

- The bond strengths of groups 1 and 2 were clinically acceptable and statistically different from groups 3 and 4. However, in the experimental groups where the varnishes were premixed with the primer and applied on the etched enamel surface and then light cured, SBS values and bracket failure rates were of such a magnitude as to make them clinically acceptable.

- Although the primer has a hydrophilic character, the antimicrobial agent in mouthwash form premixed with primer was clinically unacceptable.
- Application of hydrophilic primer, when different antimicrobial agents are mixed, significantly alters the site of failure during debonding. This may be beneficial in the clinic because less residual adhesive remains on the tooth surface.

REFERENCES

1. Bishara SE, Damon PL, Olsen ME, Jakobsen JR. Effect of applying chlorhexidine antibacterial agent on the shear bond strength of orthodontic brackets. *Angle Orthod.* 1996;66:313–316.
2. Damon PL, Bishara SE, Olsen ME, Jakobsen JR. Bond strength following the application of chlorhexidine on etched enamel. *Angle Orthod.* 1997;67:169–172.
3. Bishara SE, Vonwald L, Zamtua J, Damon PL. Effects of various methods of chlorhexidine application on shear bond strength. *Am J Orthod Dentofacial Orthop.* 1998;114:150–153.
4. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. *J Dent Res.* 1970;49:320–324.
5. Chatterjee R, Kleinberg I. Effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. *Arch Oral Biol.* 1979;24:97–100.
6. Corbett JA, Brown LR, Keene HJ, Horton IM. Comparison of Streptococcus mutans concentrations in non-banded and banded orthodontic patients. *J Dent Res.* 1981;60:1936–1942.
7. Mattingly JA, Sauer GJ, Yancey JM, Arnold RR. Enhancement of Streptococcus mutans colonization by direct bonded orthodontic appliances. *J Dent Res.* 1983;62:1209–1211.
8. Scheie AA, Arnesberg P, Krogstad O. Effects of orthodontic treatment on prevalence of Streptococcus mutans in plaque and saliva. *Scand J Dent Res.* 1984;92:211–217.
9. Vierrou AM, Manwell MA, Zameck RL, Sachdeva R, Tinanoff N. Control of Streptococcus mutans with topical fluorides in patients undergoing orthodontic treatment. *J Am Dent Assoc.* 1986; 113:644–646.
10. Gorelick L, Geiger AM, Gwinnet AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod.* 1982;81:93–98.
11. Stratemann MW, Shannon IL. Control of decalcification in orthodontic patients by daily self-administered application of a water free 0.4% SnF_2 gel. *Am J Orthod.* 1974;66:273–279.
12. Øgaard B, Larsson E, Henriksson T, Birkhed D, Bishara S. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2001; 120:28–35.
13. Øgaard B, Rølla G. Cariological aspects of treatment with fixed orthodontic appliances. Part II, new concept on cariostatic mechanism of topical fluoride. *Kieferorthopadishche Mitterlungen.* 1993;6:45–51.
14. Corbett JA, Brown LR, Keene HJ, Horton IM. Comparison of Streptococcus mutans concentrations in non-banded and banded orthodontic patients. *J Dent Res.* 1981;60:1936–1942.
15. Scheie AA, Arneberg P, Krogstad O. The effect of orthodontic treatment on the prevalence of Streptococcus mutans in plaque and saliva. *Scand J Dent Res.* 1984;92:211–217.
16. Alptekin NÖ, Uçan SU, Solmaz H, Erganiş O, Erdemir EO, Başçiftçi FA. Ortodontik tedavinin dişeti sağlığına etkisi: klinik ve mikrobiyolojik gözlemler [abstract in English]. *J Ankara University Dent Faculty.* 1999;26:167–178.
17. Lundström F, Krasse B. Streptococcus mutans and Lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur J Orthod.* 1987;9:109–116.
18. Eronat C, Alpöz AR. Effect of Cervitec® varnish on the salivary Streptococcus mutans levels in the patients with fixed orthodontic appliances [abstract 425]. *J Dent Res.* 1994;73(suppl).
19. Twetman S, Hallgren A, Petersson LG. Effect of antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. *Caries Res.* 1995;29:188–191.
20. Twetman S, Petersson LG. Efficacy of a chlorhexidine and a chlorhexidine-fluoride varnish mixture to decrease interdental levels of mutans streptococci. *Caries Res.* 1997;31:361–365.
21. Sandham HJ, Nadeau L, Phillips HI. The effect of chlorhexidine varnish treatment on salivary mutans streptococcal levels in child orthodontic patients. *J Dent Res.* 1992;71:32–35.
22. Tobias RS. Antibacterial properties of dental restorative materials. *Int Endod J.* 1988;21:155–160.
23. Petersson LG, Edwardsson S, Arends J. Antimicrobial effect of a dental varnish, in vitro. *Swed Dent J.* 1992;16:183–189.
24. Huizinga ED, Ruben JL, Arends J. Effect of an anti-microbial-containing varnish on root demineralization in situ. *Caries Res.* 1990;24:130–132.
25. Schaeken MJM, de Haan P. Effects of sustained-release chlorhexidine acetate on the human dental plaque flora. *J Dent Res.* 1989; 68:119–123.
26. Oliver RG. The effect of different methods of bracket removal on the amount of residual adhesive. *Am J Orthod Dentofacial Orthop.* 1988;93:196–200.
27. Artun J, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. *Am J Orthod.* 1984;85:333–340.
28. Hahn R, Weiger R, Netuschl L, Broch M. Microbial accumulation and vitality on different restoration materials [abstract 669]. *J Dent Res.* 1992;71.
29. Svanberg M, Mjor IA, Ørstavik D. Mutans streptococci in plaque from margins of amalgam, composite and glass-ionomer restorations. *J Dent Res.* 1990;69:861–864.
30. Shklair L, Keene HJ, Simonson LG. Distribution and frequency of Streptococcus mutans in caries-active individuals. *J Dent Res.* 1972;51:882–884.
31. Mandel ID. Antimicrobial mouth-rinses: overview and update. *J Am Dent Assoc.* 1994;125:25–105.
32. Löe H, Schiött CR. The effect of mouth rinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J Periodontol Res.* 1970;5:79–83.
33. Brightman LJ, Terezhalmy GT, Greenwell H, Jacobs M, Enlow DH. The effects of a 0.12 percent chlorhexidine gluconate mouth rinse on orthodontic patients ages 11 through 17 with established gingivitis. *Am J Orthod Dentofacial Orthop.* 1991;100:324–329.
34. Erverdi N, Acar A, İşgüden B, Kadir T. Investigation of bacteremia after orthodontic banding and debanding following chlorhexidine mouthwash application. *Angle Orthod.* 2001;71:190–194.
35. Reynolds I. A review of direct orthodontic bonding. *Br J Orthod.* 1975;2:171–178.
36. Zachrisson BU. Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am J Orthod.* 1976;69:285–300.
37. Morrow D, Wood DP, Speechley M. Clinical effect of subgingival chlorhexidine irrigation on gingivitis in adolescent orthodontic patients. *Am J Orthod Dentofacial Orthop.* 1992;101:408–413.
38. Soh LL, Newman HN, Strahan JD. Effects of subgingival chlorhexidine irrigation on periodontal inflammation. *J Clin Periodontol.* 1982;9:66–74.

39. MacAlpine R, Magnusson I, Kiger R, Crigger M, Garret S, Egelberg J. Antimicrobial irrigation of deep pockets to supplement oral hygiene instruction and root debridement. *J Clin Periodontol.* 1985;12:568–577.
40. Wieder SG, Newman HN, Strahan JD. Stannous fluoride and subgingival chlorhexidine irrigation in the control of plaque and chronic periodontitis. *J Clin Periodontol.* 1983;10:172–181.
41. Lander PE, Newcomb GM, Seymour JG, Powell RN. The antimicrobial and clinical effects of a single subgingival irrigation of chlorhexidine in advanced periodontal lesions. *J Clin Periodontol.* 1986;13:74–80.
42. Watts EA, Newman HN. Clinical effects on chronic periodontitis of a simplified system of oral hygiene including subgingival pulsated jet irrigation with chlorhexidine. *J Clin Periodontol.* 1986; 13:666–670.
43. Wennstrom JL, Heijl L, Dahlen G, Grondahl K. Periodic subgingival antimicrobial irrigation of periodontal pockets. I. Clinical observations. *J Clin Periodontol.* 1987;14:541–550.