Original Article

Does a chitosan-containing dentifrice prevent demineralization around orthodontic brackets?

Tancan Uysal[a]; Meltem Derya Akkurt[b]; Mihri Amasyali[c]; Suat Ozcan[d]; Ahmet Yagci[e]; Feridun Basak[f]; Deniz Sagdic[g]

ABSTRACT

Objective: To test the null hypothesis that there is no significant difference between the chitosan-containing and conventional nonfluoridated dentifrices in inhibition of enamel demineralization around orthodontic brackets.

Materials and Methods: Sixteen orthodontic patients who were scheduled to have extraction of four first premolars for orthodontic reasons were divided into two groups after the power of the study was estimated. Patients in the experimental group were instructed to use chitosan-containing dentifrice (AloeDent), and patients in the control group were instructed to use nonfluoridated dentifrice (Sensodyne Mint). After 60 days, the teeth were extracted and longitudinally sectioned. The demineralization was assessed by cross-sectional microhardness. The determinations were made at the bracket edge cementing limits and at occlusal and cervical points, 100 μm and 200 μm away from the edge. In all these positions, indentations were made at depths of 10, 20, 30, 50, 70, and 90 μm from the enamel surface. Analysis of variance (ANOVA) and Tukey test were used for statistical evaluation at $P < .05$ level.

Results: ANOVA showed statistically significant differences for the factors of dentifrice type, position, and depth ($P = .000$). Statistically significant differences for microhardness values between two tested dentifrices were observed up to 20 μm of depth from the enamel surface ($P < .05$). Lower microhardness values were found for nonfluoridated dentifrice. Significant microhardness differences were also determined between materials at occlusal and cervical 0 μm positions ($P < .05$). At these positions, chitosan-containing dentifrice showed lower demineralization than the control.

Conclusion: Chitosan-containing dentifrice may reduce the enamel decalcification found in patients with poor oral hygiene. The null hypothesis is rejected. (*Angle Orthod.* 2011;81:319–325.)

KEY WORDS: Chitosan; Tooth demineralization; Dental caries; Orthodontic brackets

[a] Associate Professor and Chair, Erciyes University, Faculty of Dentistry, Department of Orthodontics, Kayseri, Turkey and Visiting Professor, King Saud University, Riyadh, Saudi Arabia.

[b] Research Assistant, Gülhane Military Medical Academy, Center of Dental Sciences, Department of Pediatric Dentistry, Ankara, Turkey.

[c] Research Assistant, Gülhane Military Medical Academy, Center of Dental Sciences, Department of Orthodontics, Ankara, Turkey.

[d] Research Assistant, Gazi University, Faculty of Dentistry, Department of Conservative Dentistry and Endodontics, Ankara, Turkey.

[e] Research Assistant, Erciyes University, Faculty of Dentistry, Department of Orthodontics, Kayseri, Turkey.

[f] Professor, Gülhane Military Medical Academy, Center of Dental Sciences, Department of Pediatric Dentistry, Ankara, Turkey.

[g] Professor and Department Chair, Gülhane Military Medical Academy, Center of Dental Sciences, Department of Orthodontics, Ankara, Turkey.

Corresponding author: Dr Tancan Uysal, Erciyes Üniversitesi, Diş Hekimliği Fakültesi, Ortodonti Anabilim Dalı, 38039, Melikgazi, Kayseri, Turkey
(e-mail: tancanuysal@yahoo.com)

INTRODUCTION

Despite the advances in orthodontic materials and techniques, the development of cavitations around the brackets during treatment continues to be a problem.[1] Fixed appliances make it difficult for young patients to

maintain adequate oral hygiene during orthodontic treatment.[2] These appliances are linked to a high risk of developing white-spot lesions.[3] The prevalence of new decalcifications among orthodontic patients with fixed appliances is reported to range from 13% to 75%.[3,4] Patients with orthodontic brackets have an elevated risk of caries, and enamel lesions can occur within a month, irrespective of mechanical plaque control and whether fluoridated dentifrice is used.[3,5,6]

Several methods have been used to prevent or reduce enamel demineralization during orthodontic treatment, including fluoride application in various forms, enamel sealants, rigorous oral-hygiene regimens using glass-ionomer cement for bonding bracket and modified appliance designs.[7–9]

Chitosan and modified chitosans are interesting candidates in this respect. Chitosan, a natural linear biopolyaminosaccharide is obtained by alkaline deacetylation of chitin.[10–12] Chitin is chemically a polymeric N-acetyl-D-glucosamine and is mainly contained in the shells of crabs and shrimps.[13] Particular properties of chitosan are especially important. It has a pH of 6.3, which is suitable to buffer the oral pH value high enough to prevent the deleterious action of organic acids on the tooth surface.[14] This material is also biocompatible and biodegradable. It is positively charged and combines with the bacterial cell wall and membrane with bacteriostatic and bactericidal results.[15,16] Muzzarelli et al.[17] demonstrated that chitosan exhibit bactericidal action against several pathogens, including *Streptococcus mutans*. This is especially important since *S. mutans* is known to be the principal etiological factor of dental caries.[18]

Water-soluble, reduced chitosan was used as a mouth-rinse agent, and it displayed an antibacterial and plaque-reducing action.[19] Furthermore, recent studies have demonstrated that chewing chitosan-containing gum effectively inhibited the growth of cariogenic bacteria in saliva.[20]

It is well-known that sub-minimal inhibitory concentrations of several antibacterial compounds impair expression or production of bacterial adhesions, thus interfering with adherence to and colonization of host tissues.[21] Therefore, considering that, prevention from bacterial adhesion can easily be achieved in the oral cavity by the use of dentifrices containing these agents.

Studies in dental literature have demonstrated the positive effects of chitosan-containing materials on the pH changes in bacterial dental plaque[14] and also showed good antimicrobial effectiveness of these materials.[15–21]

In a different perspective, amorphous calcium–phosphate–containing orthodontic adhesive,[22–24] antibacterial monomer-containing adhesive,[25] and different topical agents[26] have been investigated in reducing enamel demineralization around brackets. However, no in vivo studies have been performed to investigate the efficiency of a dentifrice on enamel demineralization around orthodontic brackets.

Therefore, the aim of this study was to evaluate the in vivo effects of a chitosan-containing dentifrice in reducing enamel demineralization around orthodontic brackets and to compare the chitosan-containing dentifrice with the conventional nonfluoridated dentifrice. For these purposes, the null hypothesis assumed that there is no significant difference between the chitosan-containing and nonfluoridated dentifrices in inhibition of enamel demineralization around orthodontic brackets.

MATERIALS AND METHODS

This study was approved by the Ethical Committee on Research of the Gülhane Military Medical Academy, Ankara, Turkey. A power analysis was completed by G*Power Ver 3.0.10. (Franz Faul, Universität Kiel, Germany) software. Based on 1:1 ratio between groups, a sample size of eight patients in each group would give more than 80% power to detect significant differences with 0.40 effect size and at a significance level of $\alpha = .05$.

Inclusion Criteria

The inclusion criteria were as follows:

- Extraction of first premolars due to orthodontic reasons;
- No active caries lesions, no staining, no enamel defect, or no initial caries lesion on premolars that were planned to be investigated;
- Normal salivary flow rate (>1.0 mL/min);
- Normal buffer capacity (final pH: 6.5–7.2);
- No allergic reaction to any of the ingredients in dentifrices; and
- Regular tooth brushing habit.

Exclusion Criteria

The exclusion criteria were as follows:

- Poor oral hygiene,
- Occurrence of allergic reaction or mucosal irritation due to any of the ingredients in dentifrices, and
- Unexpected side effects.

Sixteen orthodontic patients who were scheduled to have four first premolar teeth extracted for orthodontic reasons were invited to participate in the study according to selection criteria, and all patients and their families provided informed consent.

All patients received a full-mouth cleaning to remove plaque in preparation for bonding. For evaluating the

baseline demineralization values of all selected teeth, a portable battery-powered laser fluorescence device, DIAGNOdent Pen (KaVo, Germany), was used.[22] The scores of two groups were less than 13, and this indicates that there was no demineralization; both were equivalent for caries risk.

Patients were divided into two equal groups: one experimental and one control. After oral hygiene training, patients were given one of the two dentifrices randomly:

- Group 1 (experimental) was given chitosan-containing dentifrice (AloeDent, Optima-Health Nutrition Ltd, Cardiff, Wales, UK), and
- Group 2 (control) was given nonfluoridated dentifrice (Sensodyne Mint, GlaxoSmithKline Consumer Healthcare, Brentford, UK).

Orthodontic brackets were bonded with Transbond XT (3M Unitek, Monrovia, Calif), a resin-based composite. A 37% phosphoric acid gel (3M Espe, St Paul, Minn) was used for 15 seconds. The teeth were rinsed with water for 30 seconds and dried with an oil-free source for 20 seconds. Transbond XT (3M Unitek) primer was applied to the etched surface in a thin film and not cured. Adhesive paste was applied to the bracket base (Dyna-Lok series, 3M Unitek), and the bracket was positioned on the tooth and pressed firmly into place. The excess adhesive was removed from around the bracket with a scaler, and the adhesive was light cured from the mesial and distal for 10 seconds each (total time 20 seconds). A light-emitting diode unit (Elipar Freelight 2, 3M-Espe) was used for curing the specimens for 20 seconds. All patients were instructed to brush their teeth twice a day with the given dentifrice and were advised against using any additional preparations.

For the testing procedure, 32 brackets were bonded for each group (16 upper and 16 lower first premolars in both groups). After 60 days, the teeth were extracted and stored in a refrigerator in flasks containing gauze dampened with 2% formaldehyde, pH 7.0, until the analysis. Demineralization was evaluated by cross-sectional microhardness method according to the literature.[6,22–28]

Cross-sectional Microhardness Analysis

One operator who was blinded from the group allocation carried out the microhardness analysis (Dr Ozcan). The roots of the teeth were removed with a water-cooled diamond disk. The crowns were hemisectioned vertically into mesial and distal halves with a large 15 HC wafering blade in an Isomet low-speed saw (Buehler, Lake Bluff, Ill). The hemisections were cut into a cervical portion and an occlusal portion. Both

portions were embedded in self-curing epoxy resin (Epo-Kwick, Buehler), leaving the cut face exposed. The half crown sections were polished with abrasive paper discs (320, 600, and 1200 grit) and polished with a 1-μm diamond spray and a cloth polishing disc (Buehler). A microhardness tester (HMV-700, Shimadzu, Kyoto, Japan) under a 2N load for 15 seconds was used for the microhardness analysis.

Forty-eight indentations were made in each half crown from eight positions and six depths according to the definitions of Pascotto et al.[6] On the buccal surface, indentations were made under the bracket. In the occlusal and cervical regions, indentations were made at the edge (0) of the bracket and at 100 μm and 200 μm away from it. Indentations were also made in the middle third of the lingual surface of each half crown, as another control. In all these positions, six indentations were made at 10, 20, 30, 50, 70, and 90 μm from the external surface of the enamel. The values of Vickers hardness number (VHN) found in the two half crowns were averaged.

Statistical Analysis

Data analyses were performed by using Statistical Package for Social Sciences (SPSS, Ver 13.0, SPSS Inc, Chicago, Ill). The Shapiro-Wilks normality test and the Levene variance homogeneity test were applied to the microhardness data. The data showed normal distribution, and there was homogeneity of variances between the groups.

Analysis of variance (ANOVA) was used to evaluate the effect of materials, depths from the enamel surface, positions, and their interactions. For multiple comparisons, the Tukey honestly significant difference (HSD) test was used. The statistically significant level was set at $P < .05$ level.

For evaluating the intra- and interobserver agreement, the microhardness measurements were done by two investigators using the same instrument at two separate times, and Cohen's kappa scores were determined.

RESULTS

The kappa scores for the assessment of intra- and interobserver agreement were higher than 0.80, which implies substantial agreement between the observers.

ANOVA showed statistically significant differences for the factors of dentifrice type, position, and depth ($P = .000$). The interactions (dentifrice type/position, dentifrice type/depth, position/depth and dentifrice type/position/depth) were also statistically significant ($P = .000$) (Table 1).

Descriptive statistics and multiple comparisons of microhardness for chitosan-containing and conven-

Table 1. Statistical Comparisons and the Results of ANOVA

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Sig
Dentifrice type	152,786.418[a]	1	168,324.316	1,872.564	.000*
Position	303,920.602	7	43,564.367	617,065	.000*
Depth	1,458,910.878	5	299,996.195	4,595.055	.000*
Dentifrice type * position	202,290.541	7	2,998.987	566,558	.000*
Dentifrice type * depth	1,431,462.640	5	23,292.528	400,303	.000*
Position * depth	293,943.671	35	7,834.710	118,451	.000*
Dentifrice type * position * depth	188,748.640	35	5,298.689	78,978	.000*

[a] Adjusted R squared = 0.945.
* Statistically significant ($P < .05$).

tional nonfluoridated dentifrice at different depths from the enamel surface are shown in Table 2. The interaction between dentifrice type and depth had significant differences at the depths of 10 μm and 20 μm from the enamel surface. Less demineralization was found on enamel around the brackets for the experimental group compared with the control group.

All descriptive statistics and multiple comparisons of microhardness of two dentifrice systems at different observation positions are shown in Table 3. Statistically significant microhardness differences were determined for the two tested groups ($P < .05$). Chitosan-containing dentifrice showed higher microhardness (occlusal 0 μm: 319.356 VHN; cervical 0 μm: 320.156 VHN) than the conventional dentifrice (occlusal 0 μm: 279.214 VHN; cervical 0 μm: 281.451 VHN).

All descriptive statistics and multiple comparisons of microhardness of two dentifrice systems and positions at depths of 10 μm and 20 μm are shown in Table 4. The highest demineralization values were determined at occlusal and cervical 0 μm positions for the control group at 10 μm and 20 μm depth.

According to these results, the null hypothesis of the present study is rejected.

DISCUSSION

Present findings showed that the use of chitosan-containing dentifrice during 60 days of orthodontic treatment was able to prevent demineralization of enamel, and its use can be suggested in prevention of white-spot lesions. To our knowledge, this study is the first to compare the use of a specific dentifrice with a conventional nonfluoridated dentifrice by evaluating condition with microhardness testing enamel demineralization around orthodontic brackets in an in vivo condition, by microhardness testing.

In the past, to use fewer patients and for ethical considerations, preventive effects of various products such as fluoride-releasing materials against demineralization were investigated by using a split-mouth study design.[27] A split-mouth design was unsuitable for this investigation. Moreover oral hygiene characteristics are not always homogeneously distributed over the within-patient experimental units, and this heterogeneity can reduce the efficiency of split-mouth designs. As suggested by Pascotto et al.,[6] the current experimental design was chosen instead of the split-mouth technique to avoid the carry-across effect. Therefore, subjects were divided into two groups. The baseline clinical, radiologic, salivary, and laser fluorescence assessments were done for standardization. It was determined that all patients in both groups were equivalent with regard to caries risk or demineralization activity.

Instead of in vitro studies with extracted teeth, our model had several advantages[27]: the development of the caries lesions was studied in vital teeth; minimal patient cooperation was required; no special diet was required; and because the protected enamel surface allowed the accumulation of thick plaque, no other site was at risk of caries with this procedure.

Table 2. Descriptive Statistics and Multiple Comparisons of Microhardness (VHN) for Chitosan-containing and Conventional Dentifrice Systems at Different Depths From the Enamel Surface

Interaction of Dentifrice Type/Depth, μm	Nonfluoridated Dentifrice		Chitosan-containing Dentifrice		Multiple Comparisons[a]
	Mean	SD	Mean	SD	
10	271.280	7.456	303.123	8.456	*
20	287.235	8.342	309.937	9.662	*
30	295.691	8.741	314.108	9.019	NS
50	322.412	9.456	324.672	8.451	NS
70	331.123	8.392	330.178	7.592	NS
90	345.431	9.014	346.989	7.491	NS

[a] Tukey HSD test. NS indicates not significant.
* Statistically significant ($P < .05$).

Table 3. Descriptive Statistics and Multiple Comparisons of Microhardness (VHN) of Two Dentifrice Systems at Different Observation Positions

Interaction of Dentifrice Type/Position	Nonfluoridated Dentifrice		Chitosan-containing Dentifrice		Multiple Comparisons[a]
	Mean	SD	Mean	SD	
Occlusal 200 μm	315.349	18.659	318.435	18.234	NS
Occlusal 100 μm	315.795	23.126	319.545	15.941	NS
Occlusal 0 μm	279.214	22.945	319.356	16.724	*
Under bracket	325.312	19.276	323.971	17.465	NS
Cervical 0 μm	281.451	24.252	320.156	18.297	*
Cervical 100 μm	314.601	23.810	317.600	17.567	NS
Cervical 200 μm	319.822	20.183	320.412	18.278	NS
Lingual	325.603	17.108	324.101	15.240	NS

[a] NS indicates not significant.
* Statistically significant ($P < .05$).

The demineralization values of enamel under two internal controls (under the bracket and at the lingual surface) bonded to the two types of teeth were used to evaluate the effect of acid etching and enamel demineralization.[6]

In this study, the mineral loss was assessed by cross-sectional microhardness, an accepted analytic method. This method was preferred to evaluate demineralization and caries because a strong correlation coefficient ($r = 0.91$) was reported by Featherstone and coworkers[28] between enamel microhardness scores and the percentage of mineral loss in the caries lesions.

Pascotto et al.[6] observed reduced enamel hardness in the cervical region of the bracket compared with that in the occlusal area. These findings were similar to current findings. In vivo, the explanation for this may be that the greater dental plaque accumulation and patient difficulty in cleaning this area. In vitro, the explanation would be less mineralization and higher carbonate on the cervical surface than in the occlusal region.[6] Interestingly in the present study, different from the previous findings,[6,27,28] similar mineral loss was observed at the cervical and the occlusal region at 0 μm positions. Statistically significant microhardness differences were determined at these regions between the tested materials. Control group showed lower hardness values that indicate more mineral loss than the tested materials.

While others have reported that the demineralization of the enamel around orthodontic brackets can extend as far as 75 μm below the enamel surface, Gorton and Featherstone[1] and Pascotto et al.[6] reported that enamel demineralization extended to only 30 μm from the enamel surface in vivo. They permitted their subjects to brush their teeth, which most probably removed some or all of the plaque and microorganisms accountable for demineralization. In this study, for extensive and controlled assessment, the indentations we made were at 10, 20, 30, 50, 70, and 90 μm from the external surface of the enamel to observe mineral changes at the outermost part of the enamel. The

Table 4. Descriptive Statistics and Multiple Comparisons of Microhardness (VHN) of Two Dentifrice Systems and Positions at Depth of 10 and 20 μm

Depth, μm	Position	Nonfluoridated Dentifrice				Chitosan-containing Dentifrice				Multiple Comparisons[a]
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	
10	Occlusal 200 μm	301.222	8.211	279.174	312.979	305.319	7.576	288.594	317.841	NS
	Occlusal 100 μm	292.602	9.834	271.238	309.683	313.108	9.627	299.114	332.841	NS
	Occlusal 0 μm	212.733	8.931	189.264	225.479	299.653	7.582	278.624	313.831	*
	Under bracket	314.371	8.596	286.914	325.469	313.578	8.985	299.924	332.721	NS
	Cervical 0 μm	182.583	9.284	157.584	194.539	295.824	9.023	279.374	317.051	*
	Cervical 100 μm	285.135	10.680	251.264	302.665	305.130	8.900	292.494	328.191	NS
	Cervical 200 μm	303.090	9.944	282.954	316.299	304.010	8.548	283.524	317.401	NS
	Lingual	319.483	8.881	299.954	333.239	320.403	8.607	305.264	338.401	NS
20	Occlusal 200 μm	315.734	6.447	297.964	329.389	316.672	6.559	299.504	328.461	NS
	Occlusal 100 μm	316.024	9.346	283.324	329.255	324.870	7.405	308.684	344.541	NS
	Occlusal 0 μm	242.983	8.698	220.264	262.959	319.281	7.861	308.954	342.761	*
	Under bracket	325.657	9.299	308.484	340.259	321.348	9.855	304.924	338.651	NS
	Cervical 0 μm	215.938	10.941	179.924	233.409	308.966	7.199	290.434	322.011	*
	Cervical 100 μm	300.775	8.929	283.854	317.199	317.781	9.638	304.964	332.101	NS
	Cervical 200 μm	315.883	9.394	298.164	330.199	313.496	9.803	298.224	332.351	NS
	Lingual	325.240	8.372	308.424	340.599	322.388	8.228	308.954	342.761	NS

[a] NS indicates not significant.
* Statistically significant ($P < .05$).

results of the current study demonstrate that, up to 20-μm depth from enamel surface, the microhardness values for nonfluoridated dentifrice were lower than for the experimental dentifrice, and the difference was statistically significant.

Different from our findings, de Moura et al.[27] found lesion depths up to 70 μm from the enamel surface. Our results showed that demineralization existed only up to 20 μm of depth from the enamel surface. This could be explained by the experimental model used; they allowed more plaque accumulation and impaired its removal by tooth brushing. Multiple comparisons of demineralization for dentifrice type and position at the 10 μm depth from the enamel surface showed statistically significant differences at all positions in both the cervical and occlusal margins evaluated on the buccal surface.

Orthodontic attachments make a patient's dental hygiene more difficult, and the accumulation of plaque is easier around the brackets. Our results confirmed this by showing the lower demineralization values at the occlusal and cervical margins at 0 μm positions (Table 3).

To date, the use of chitosan-containing chewing gum and mouth rinse has been found to be an effective method for preventing demineralization of enamel. Hayashi et al.[20] have reported that chitosan-containing chewing gum was more effective than chitosan-containing mouth rinse. Hayashi et al.[20] have also reported that chitosan released from the gum base could be preserved at the bacteriostatic level in the saliva. The antimicrobial activity of chitosan is dependent on its molecular weight and degree of deacetylation.[29] Fujiwara et al.[15] have proved that the highly deacetylated and lower molecular chitosan showed bactericidal activity. Present findings suggested that the supplementation of chitosan to dentifrice is an effective method to control the demineralization of enamel around brackets.

CONCLUSION

- Chitosan-containing dentifrice may reduce the enamel decalcification found in patients with poor oral hygiene.

REFERENCES

1. Gorton J, Featherstone JDB. In vivo inhibition of demineralization around orthodontic brackets. *Am J Orthod Dentofacial Orthop*. 2003;123:10–14.
2. Tanna N, Kao E, Gladwin M, Ngan PW. Effects of sealant and self-etching primer on enamel decalcification. Part I: an in-vitro study. *Am J Orthod Dentofacial Orthop*. 2009;135: 199–205.
3. Wenderoth CJ, Weinstein M, Borislow AI. Effectiveness of a fluoride-releasing sealant in reducing decalcification during orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 1999;116:629–634.
4. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod Dentofacial Orthop*. 1982;81:93–98.
5. Øgaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod Dentofacial Orthop*. 1988;94:68–73.
6. Pascotto RC, Navarro MFL, Capelozza Filho L, Cury JA. In vivo effect of a resin-modified glass ionomer cement on enamel demineralization around orthodontic brackets. *Am J Orthod Dentofacial Orthop*. 2004;125:36–41.
7. Ghiz MA, Ngan P, Kao E, Martin C, Gunel E. Effects of sealant and self-etching primer on enamel decalcification. Part II: an in-vivo study. *Am J Orthod Dentofacial Orthop*. 2009;135:206–213.
8. Chung CK, Millett DT, Creanor SL, Gilmour WH, Foye RH. Fluoride release and cariostatic ability of a compomer and a resin-modified glass ionomer cement used for orthodontic bonding. *J Dent*. 1998;26:533–538.
9. Fricker JP. Bonding and debonding with a light-activated resin-modified glass-ionomer cement. *Aust Orthod J*. 1996; 14:78–80.
10. Muzzarelli RA. Chitins and chitosans as immuneadjuvants and non-allergenic drug carriers. *Mar Drugs*. 2010;21: 292–312.
11. Roberts GAF. *Chitin Chemistry*. Houndmills, UK: MacMillan Press; 1992.
12. Rinaudo M, Domard A. In: Skjak-Brack G, Anthonsen T, Sandford P, eds. *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications*. London, UK: Elsevier Applied Science; 1989:1–37.
13. Petri DF, Donegá J, Benassi AM, Bocangel JA. Preliminary study on chitosan modified glass ionomer restoratives. *Dent Mater*. 2007;23:1004–1010.
14. Shibasaki K, Sano H, Matsukubo T, Takaesu Y. Effects of low molecular chitosan on pH changes in human dental plaque. *Bull Tokyo Dent Coll*. 1994;35:33–39.
15. Fujiwara M, Hayashi Y, Ohara N. Inhibitory effect of water-soluble chitosan on growth of *Streptococcus mutans*. *New Microbiol*. 2004;27:83–86.
16. Vishu Kumar AB, Varadaraj MC, Gowda LR, Tharanathan RN. Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and pronase, and their bactericidal action against *Bacillus cereus* and *Escherichia coli*. *Biochem J*. 2005;391:167–175.
17. Muzzarelli R, Tarsi R, Filippini O, Giovanetti E, Biagini G, Varaldo PE. Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrob Agents Chemother*. 1990;34:2019–2023.
18. Jenkinson HF. Adherence and accumulation of oral streptococci. *Trends Microbiol*. 1994;2:209–212.
19. Bae K, Jun EJ, Lee SM, Paik DI, Kim JB. Effect of water-soluble reduced chitosan on *Streptococcus mutans*, plaque regrowth and biofilm vitality. *Clin Oral Investig*. 2006;10: 102–107.
20. Hayashi Y, Ohara N, Ganno T, et al. Chewing chitosan-containing gum effectively inhibits the growth of cariogenic bacteria. *Arch Oral Biol*. 2007;52:290–294.
21. Zhanel GG, Nicolle LE. Effect of subinhibitory antimicrobial concentrations (sub-MICs) on in-vitro bacterial adherence to uroepithelial cells. *J Antimicrob Chemother*. 1992;29: 617–627.
22. Uysal T, Amasyali M, Koyuturk AE, Sagdic D. Efficiency of amorphous calcium phosphate-containing orthodontic composite and resin modified glass ionomer on demineralization evaluated by a new laser fluorescence device. *Eur J Dent*. 2009;3:127–134.

23. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Akyol M, Sagdic D. In vivo effects of amorphous calcium phosphate–containing orthodontic composite on enamel demineralization around orthodontic brackets. *Aust Dent J.* 2010;55:285–291.

24. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Sagdic D. Amorphous calcium phosphate-containing orthodontic composites. Do they prevent demineralisation around orthodontic brackets? *Aust Orthod J.* 2010;26:10–15.

25. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Sagdic D. Effect of antibacterial monomer–containing-adhesive on enamel demineralization around orthodontic brackets. In vivo study. *Am J Orthod Dentofacial Orthop.* In press.

26. Uysal T, Amasyali M, Koyuturk AE, Ozcan S. Effects of different topical agents on enamel demineralization around orthodontic brackets. In vivo and in vitro study. *Aust Dent J.* 2010;55:268–274.

27. de Moura MS, de Melo Simplício AH, Cury JA. In-vivo effects of fluoridated antiplaque dentifrice and bonding material on enamel demineralization adjacent to orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 2006;130: 357–363.

28. Featherstone JBD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesion by quantitative microradiography and microhardness profiles. *Caries Res.* 1983;17:385–391.

29. No HK, Park NY, Kee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int J Food Microbiol.* 2002;74:65–72.