ORIGINAL RESEARCH

Marginal Adaptation of Class V Restorations with Current-Generation Dentin-Bonding Agents: Effect of Different Dentin Surface Treatments

[1]Jessica Patrícia Cavalheiro, [2]Mateus Rodrigues Tonetto, [3]Álvaro Henrique Borges, [4]Matheus Coelho Bandéca
[5]Reidson Stanley Soares dos Santos, [6]Edson Alves de Campos, [7]José Roberto Cury Saad, [8]Andréa Abi Rached
Dantas, [9]Marcelo Ferrarezi de Andrade

ABSTRACT

This study sought to assess the use of chlorhexidine with several excipients as a dentin surface treatment and its effect on marginal adaptation of class V restorations with current-generation dentin bonding agents. A total of 120 human third molars were selected and allocated into 12 groups, with standardized buccal class V restorations randomly divided into preconditioned dentin rinsed with: water; water + chlorhexidine; ethanol; or ethanol + chlorhexidine. After rinsing of dentin (previously conditioned with 35% phosphoric acid) with the test solutions, the Adper single bond 2, prime and bond 2.1, and Excite bonding systems were applied randomly. Restorations were performed with Filtek™ Z350 XT composite resin. The resulting specimens were subjected to thermal and mechanical load cycling. Quantitative analysis of marginal adaptation was performed on epoxy replicas by means of scanning electron microscopy. Results were assessed by means of the Kruskal-Wallis test (percentages of continuous margins) and Wilcoxon test (differences between percentages of continuous margins before and after thermal cycling and mechanical loading), at a significance level of $p < 0.05$. Outcomes in the chlorhexidine-treated groups were not superior to those obtained with other treatments.

Keywords: Dentin-bonding agents, Marginal adaptation, Chlorhexidine, Ethanol, Scanning electron microscopy.

[1]Undergraduate Student, [2-9]Professor

[1]Department of Dentistry, UNESP, Araraquara, SP, Brazil

[2,3]Department of Postgraduation in Integrated Dental Sciences University of Cuiaba, MT, Brazil

[4,5]Department of Postgraduation in Dentistry, CEUMA University, São Luis, MA, Brazil

[6-9]Department of Restorative Dentistry, UNESP, Araraquara SP, Brazil

Corresponding Author: Mateus Rodrigues Tonetto, Professor Department of Postgraduation in Integrated Dental Sciences, AV Pio Correa Pinheiro, No. 651, Apto 31-B, Melhado, 14807-031 Araraquara, SP, Brazil, e-mail: mateus.tonetto@foar.unesp.br

INTRODUCTION

Dentin bonding agents are widely employed in modern dentistry. In view of this broad applicability, research into their properties is extremely important. Bonding of resin to dentin occurs by means of infiltration and polymerization of hydrophilic resins into the collagen mesh exposed by acid treatment of dentin, forming a hybrid layer.[1] Several authors have demonstrated degradation of the resin-dentin bond,[2-6] and the durability of resin restorations is currently a subject of great interest to study of the behavior of adhesive systems. Although the mechanism, whereby the adhesive-dentin inter-face deteriorates, has yet to be fully elucidated, dentin matrix metalloproteinases (MMPs), a family of proteolytic enzymes capable of degrading the organic matrix of demineralized dentin, are known to act on collagen fibrils at the base of the hybrid layer, which are exposed due to discrepancies between the depth of acid demineralization of dentin and monomer infiltration during bonding procedures.[7,8]

Chlorhexidine is widely used as an antimicrobial agent. Studies have suggested that the technique of chlorhexidine treatment of conditioned dentin prior to use of bonding agents may inhibit MMPs and, consequently, prevent the degradation of collagen fibrils at the resin-dentin bond. A 2% aqueous solution of chlorhexidine, when used as an adjuvant to the process of polymer adhesion to the dentin substrate, does not interfere negatively with immediate bonding perfor-mance; however, the resin component remains vulnerable to hydrolytic degradation.[9,10]

Hydrophobic monomers, exhibiting greater resistance to hydrolytic degradation and, possibly, to demineralized dentin infiltration, have been widely investigated.[11-14] Due to the presence of water in dentin, adhesive systems contain hydrophilic components. As bonding techniques have been simplified, the latest generations of bonding agents have become highly hydrophilic; as a consequence of water absorp-tion, the rate of degradation increases, as do mechanical

properties that may affect long-term survival of the adhesive-restoration interface.[15,16] To address this issue, a new technique known as 'ethanol-wet bonding'[17] was developed, in which ethanol is used instead of water. Theoretically, use of this technique would yield a less hydrophilic environment, keeping the collagen matrix expanded and allowing monomers to permeate through to the interfibrillary spaces. Some aspects have yet to be validates, such as the use of a combination of ethanol and chlorhexidine, as both solutions have been shown to produce favorable outcomes in terms of the resin-dentin bond.

Furthermore, the use of chlorhexidine on demineralized dentin may increase its surface free energy to levels resembling those of enamel,[18,19] thus enhancing the wettability of demineralized dentin by adhesive systems, which could explain the higher immediate bond strength values observed in chlorhexidine-treated groups.[20,21] Studies are currently being conducted to assess whether interventions designed to minimize the effects of enzymatic and hydrolytic degradation of the resin-dentin bond (whether in combination or alone) can interfere with the formation and mechanical stability of the resin-dentin bond in carious teeth.

In view of the high rate of failure of dental restorations, which is more common in the posterior teeth than in the anterior teeth,[22,23] dental practitioners must be mindful of the conditions to which restorations are exposed, including ambient temperature and mechanical load cycles associated with mastication[24-26] and understand the contributions of each potential mechanism of degradation so as to ensure a better resin-dentin bond and a stable adhesive interface.[27]

Within this context, the present article seeks to investigate the influence of chlorhexidine, when used as an adjuvant to the process of bonding to the dentin substrate on marginal adaptation after thermal cycling and scanning electron microscope analysis.

MATERIALS AND METHODS

A total of 120 noncarious human third molars were extracted and stored in 1% thymol at 4°C until use. All patients were provided information on the study and gave written informed consent for use of their teeth. The study was approved by the UNESP Araraquara School of Dentistry Research Ethics Committee with judgement number 52/11.

The teeth were allocated into 12 groups of 10 specimens each. A standardized class V cavity (depth 2.0 mm, cervico-incisal length 2.0 mm, mesiodistal length 4.0 mm) was created on the buccal surface of each specimen with the aid of a round diamond bur. Burs were replaced after preparation of four cavities.

Surface Treatment

Prepared teeth were randomly divided into subgroups according to the dentin surface treatment applied. All enamel and dentin surfaces were conditioned with 35% phosphoric acid (Ultradent Products Inc., South Jordan, Utah, USA) for 30 and 15 seconds respectively, rinsed in water for 10 seconds, and dried with absorbent paper.

- *Conditioned dentin rinsed with water*: Conditioned dentin was kept moist and the bonding agent was applied without any further surface treatment.
- *Conditioned dentin rinsed with chlorhexidine in water*: Conditioned dentin was treated with 20 µl of a solution of 1% chlorhexidine diacetate in water (0.25 gm/25 ml), which was kept in place for 60 seconds and then dried off with absorbent paper.
- *Conditioned dentin rinsed with ethanol*: Conditioned dentin was treated with 20 µl of 100% anhydrous ethanol (JT Baker, Mallinckrodt SA, Xalostoc, Mexico), which was kept in place for 60 seconds and then dried off with absorbent paper.
- *Conditioned dentin rinsed with chlorhexidine in ethanol*: The smear layer-coated dentin surface was conditioned and, under similar moisture conditions, treated with 20 µl of a compounded solution of 1% chlorhexidine diacetate in anhydrous ethanol (0.19 gm/25 ml). The solution was kept in place for 60 seconds and then dried off with absorbent paper.

Bonding Procedure

After treatment of conditioned dentin with the test solutions, the teeth were randomly subdivided by surface treatment type and the dentin adhesive systems Adper single bond 2 (3M ESPE, St Paul, MN, USE), prime and bond 2.1 (Dentsply, Milford, DE, USA), and Excite (IvoclarVivadent, Schaan, Liechtenstein) were applied randomly in accordance with manufacturer instructions.

After the bonding procedure, cavities were restored with Filtek™ Z350 XT composite resin, shade A3 (3M ESPE, St Paul, MN, EUA). The composite resin was placed incrementally into the cavity—one portion onto the axial wall, one onto the mesial wall, and one onto the distal wall and each increment was light-cured for 20 seconds.

Following curing, restorations were finished and polished with Sof-Lex™ Pop-On tooth polishing discs (3M ESPE) and a silicon carbide brush. The restored teeth were then stored in water at 37°C in a dark area.

After specimens had been in storage for 1 week, impressions of the external margins of each restoration were obtained using a vinyl polysiloxane impression material

(President light-body, Coltène-Whaledent AG, Altstätten, Switzerland) and epoxy resin replicas were made (Epofix, Stuers, Rodovre, Denmark). The original specimens were then subjected to combined thermal and mechanical loading in an artificial masticator. Thermal cycling consisted of 3,000 cycles in water at temperatures of 5 and 50°C, with specimens remaining for 2 minutes at each temperature extreme.

After loading, impressions of the external margins of each restoration were once again obtained and epoxy resin replicas made using the same procedure described above.

The resulting specimens were sputter-coated with gold in a BAL-TEC SCD 050 Sputter Coater, and quantitative analysis of the external margins was performed by means of scanning electron microscopy (Jeol JSM-T330A Scanning Microscope).

RESULTS

The Shapiro-Wilk test revealed a non-normal distribution of data in some groups, and Levene's test showed heterogeneity of variances. Therefore, nonparametric tests were used for all statistical analyses.

To assess marginal adaptation, differences in percentages of 'continuous margins' were evaluated by means of the Kruskal-Wallis test with Dunn's post-hoc test. Independent tests were conducted for each dentin adhesive system, before and after thermal cycling. The Wilcoxon test was used to assess the differences between percentages of continuous margins before and after thermal cycling for each combination of dentin surface treatment and bonding agent. The significance level was set at 5%.

Results are shown in Table 1. In specimens restored with the excite adhesive system, there were no differences in dentin or enamel continuous margin between the distinct surface treatment subgroups. However, on analysis of the effects of thermal cycling, significant differences in enamel were observed in specimens treated with ethanol and in those treated with chlorhexidine. Significant differences in dentin were found in the chlorhexidine and ethanol + chlorhexidine-treated groups.

In specimens restored with the Adper single bond system, there were no differences in enamel between any of the surface treatment subgroups (Figs 1A and B). In dentin, significant differences were found in the ethanol, ethanol + chlorhexidine, and chlorhexidine-treated groups, both before and after thermal cycling.

The greatest changes in external margin after thermal cycling were found in specimens restored with the prime and bond 2.1 system, both in dentin and in enamel (Figs 1C and D).

DISCUSSION

As shown in Table 1, in specimens restored with the Excite adhesive system, there were significant differences before and after thermal cycling in the ethanol-treated and chlorhexidine-treated groups in enamel and in the chlorhexidine and ethanol + chlorhexidine-treated groups in dentin. Analysis of findings for the aforementioned groups in enamel showed that median values had relatively narrow ranges, in the region of 93.50. However, comparative analysis of results that differed significantly *vs* those that did not in the

Table 1: Median (± SD) percentages of continuous margins in enamel and dentin achieved with the Excite, Adper single bond 2, and prime and bond 2.1 adhesive systems, analyzed individually, taking into account each dentin surface treatment and combined thermal cycling and mechanical loading

Bonding agent	Treatment	Continuous margin, enamel (%)		Continuous margin, dentin (%)	
		Precycling	*Postcycling*	*Precycling*	*Postcycling*
Excite	Water	98.50 (± 2.28)[Aa]	93.65 (± 2.81)[Aa]	92.95 (± 1.10)[Aa]	94.05 (± 5.49)[Aa]
	Ethanol	99.00 (± 2.23)[Aa]	93.50 (± 8.52)[Ab]	87.60 (± 21.57)[Aa]	71.50 (± 21.43)[Aa]
	CLX	95.50 (± 2.28)[Aa]	93.95 (± 2.34)[Ab]	88.50 (± 6.25)[Aa]	78.55 (± 4.85)[Ab]
	Ethanol + CLX	99.00 (± 2.09)[Aa]	93.50 (± 6.73)[Aa]	92.30 (± 8.38)[Aa]	82.40 (± 16.05)[Ab]
Adper single bond	Water	96.15 (± 7.13)[Aa]	86.80 (± 7.15)[Aa]	86.15 (± 8.11)[Aa]	79.55 (± 10.16)[ABa]
	Ethanol	97.65 (± 6.07)[Aa]	93.40 (± 11.72)[Aa]	91.50 (± 22.33)[Aa]	70.20 (± 12.61)[Ba]
	CLX	98.50 (± 1.94)[Aa]	96.00 (± 1.33)[Aa]	94.10 (± 4.15)[Aa]	87.60 (± 2.01)[Ab]
	Ethanol + CLX	99.35 (± 1.59)[Aa]	93.60 (± 7.30)[Aa]	84.60 (± 11.96)[Aa]	75.75 (± 6.61)[Ba]
Prime and bond	Water	100.0 (± 0.87)[Aa]	83.50 (± 5.24)[Ab]	96.00 (± 5.03)[Aa]	83.25 (± 11.42)[Aa]
	Ethanol	98.00 (± 0.99)[ABa]	84.25 (± 7.59)[Ab]	97.50 (± 6.13)[Aa]	70.50 (± 4.65)[Ab]
	CLX	95.85 (± 8.45)[Ba]	83.45 (± 13.09)[Ab]	94.75 (± 14.85)[ABa]	68.25 (± 2.213)[Ab]
	Ethanol + CLX	89.65 (± 5.81)[Ba]	79.45 (± 7.17)[Ab]	73.75 (± 13.99)[Ba]	62.05 (± 10.74)[Ab]

CLX: Chlorhexidine; Values sharing the same superscript uppercase letters are not significantly different from each other (Kruskal-Wallis test with Dunn's post-hoc test, p < 0.05) in terms of different materials. Values sharing the same superscript lowercase letters are not significantly different from each other (Wilcoxon test with Dunn's post-hoc test, p < 0.05) in terms of the effects of thermomechanical cycling on each material and surface (enamel or dentin)
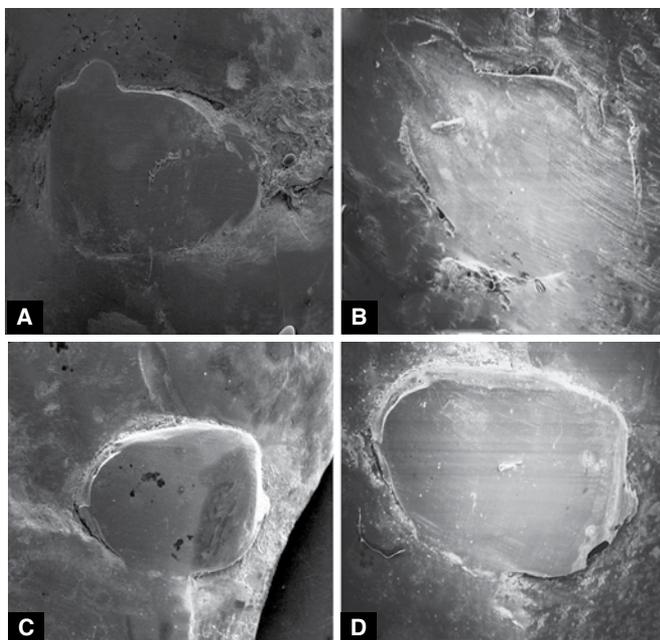
Figs 1A to D: Representative scanning electron micrographs of study specimens: (A) Treatment: ethanol + chlorhexidine; bonding agent: Adper single bond 2; precycling (B) Treatment: ethanol + chlorhexidine; bonding agent: Adper single bond 2; postcycling (C) Treatment: ethanol + chlorhexidine; bonding agent: prime and bond 2.1; precycling: (D) Treatment: ethanol + chlorhexidine; bonding agent: prime and bond 2.1; postcycling

dentin groups suggests that the combination of chlorhexidine and water may have somehow compromised the interaction between the excite bonding agent and the dentin surface. Chlorhexidine diacetate may undergo adsorption at the dentin surface, which would be a desirable feature from the standpoint of prolonged protection. However, adsorption of chlorhexidine to exposed collagen can affect surface wetting properties, which may have had a deleterious effect on the excite adhesive system.[13] Individual comparisons of the different surface treatments in enamel and in dentin showed no significant differences.

The greatest changes in external margins after thermal cycling were observed in the prime and bond 2.1 group, both in dentin and in enamel, with the exception of the water-treated dentin subgroup. This poorer performance of the prime and bond 2.1 system is explained by the presence of acetone in its chemical composition. Due to its solubility pattern, acetone has a lower hydrogen-bonding capacity than that found among peptides. When applied to an expanded matrix in the presence of water, solvents with such lower capacity do not produce re-expansion of the matrix, but rather collapse,[9,17] which would explain the poorer performance of acetone-based adhesive systems as compared with ethanol/water-based ones.[6,9] Comparative analysis of the various surface treatments employed showed a significant impact on enamel continuous margin in the chlorhexidine and ethanol + chlorhexidine-treated groups and significant effects on dentin continuous margin in the ethanol + chlorhexidine groups. However, comparison among surface treatments after thermal cycling showed no significant differences between groups, including the aforementioned ones, which indicates preservation of continuous margins both in enamel and in dentin.

With the Adper single bond 2 system, there were no significant differences between the various surface treatments before and after thermal cycling, i.e. enamel margin integrity was preserved. These results are explained by the solvents of the Adper single bond 2 bonding system: water and ethanol, which have a surface tension of 72.0 and 22.4 Mn/m respectively. The greater surface tension of water reduces the ability of this solvent to penetrate into the substrate as compared with bonding agents containing ethanol only. The solubility of ethanol is higher, and it is thus able to re-expand or maintain expansion of the collagen matrix, although more slowly and to a lesser degree as compared with water.[9,17] These relationships among bonding agents, their solvents (and water), and the demineralized matrix explain, e.g. the superior performance of ethanol/water-based adhesives as compared with acetone-based bonding agents.

Analysis of the effects of the Adper single bond 2 system on the dentin substrate showed statistically significant differences in chlorhexidine-treated specimens, before (94.10 ± 4.15) and after cycling (87.60 ± 2.01). Despite this difference, median values were higher as compared with those of other surface treatment groups and effects of thermal cycling. In previous studies, chlorhexidine treatment of dentin demineralized with phosphoric acid prior to application of a bonding agent led to preservation of the integrity of collagen within the hybrid layer over time[5] and acted as a metalloproteinase inhibitor. Consequently, it might help maintain the stability of adhesive binding to dentin over time. Therefore, *in vitro* and *in vivo* studies have demonstrated that use of chlorhexidine after acid conditioning and before bonding agent application successfully enhances the durability of the resin-dentin bond.[1,5] As the efficiency of infiltration of adhesive resin into the demineralized zone is unpredictable, practitioners must anticipate that, under normal bonding conditions, cavities will always contain exposed collagen fibrils susceptible to the effects of MMPs. Within this context, chlorhexidine acted as an additional mechanism to protect the resin-dentin bond.

The results of this project are somewhat inconclusive with respect to the use of chlorhexidine, as it was not found to be superior on comparison with other dentin surface treatments. Nevertheless, the stability of chlorhexidine-treated teeth has been reported in the literature.[1,5]

REFERENCES

1. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, Mai S, Pashley DH. In vivo chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive. Oper Dent 2009;34(4): 379-383.

2. Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzaga CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. J Dent 2009;37(2):108-114.

3. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, V anherle G. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82(2):136-140.

4. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, Ruggeri A Jr, Tay FR, Dorigo Ede S, Pashley DH. Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. Dent Mater 2010;26(4):320-325.

5. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86(6):529-533.

6. Reis AF, Giannini M, Pereira PNR. Effects of a peripheral enamel bond on the long-term effectiveness of dentin bonding agents exposed to water in vitro. J Biomed Mater Res Part B: Appl Biomater 2008;85B:10-17.

7. Eliades G, Vougiouklakis G, Palaghias G. Heterogeneous distribution of single-bottle adhesive monomers in the resin-dentin interdiffusion zone. Dent Mater 2001;17(4):277-283.

8. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res 2002;62(3):447-456.

9. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83(3):216-221.

10. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, Breschi L, Foulger S, Pashley DH. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. J Biomed Mater Res B Appl Biomater 2009;90(1):373-380.

11. Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, Tay FR. Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. J Biomed Mater Res A 2008;84(1):19-29.

12. Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, Tay KC. Bonding BisGMA to dentin: a proof of concept for hydrophobic dentin bonding. J Dent Res 2007;86(11):1034-1039.

13. Cadenaro M, Breschi L, Rueggeberg FA, Agee K, Di Lenarda R, Carrilho M, Tay FR, Pashley DH. Effect of adhesive hydrophilicity and curing time on the permeability of resins bonded to water vs ethanol-saturated acid-etched dentin. Dent Mater 2009;25(1):39-47.

14. Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, Watson TF, Osorio R. Resin-dentin bonds to EDTA-treated vs acid-etched dentin using ethanol wet-bonding. Dent Mater 2010;26(4):368-379.

15. Ye Q, Spencer P, Wang Y, Misra A. Relationship of solvent to the photopolymerization process, properties, and structure in model dentin adhesives. J Biomed Mater Res A. 2007;80(2): 342-350.

16. Ye Q, Wang Y, Spencer P. Nanophase separation of polymers exposed to simulated bonding conditions. J Biomed Mater Res B ApplBiomater 2009;88(2):339-348.

17. Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, Donnelly A, García-Godoy F. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007;20(1):7-20.

18. Perdigão J, Denehy GE, Swift Jr EJ. Effects of chlorhexidine on dentin surfaces and shear bond strengths. Am J Dent. 1994;7(2): 81-84.

19. Perdok JF, van der Mei HC, Genet MJ, Rouxhet PG, Busscher HJ. Elemental surface concentration ratios and surface free energies of human enamel after application of chlorhexidine and adsorption of salivary constituents. Caries Res 1989;23(5):297-302.

20. de Castro FL, de Andrade MF, Duarte Júnior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. J Adhes Dent 2003;5(2):129-138.

21. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86(6):529-533.

22. Tortopidis D, Lyons MF, Baxendale RH, Gilmour WH. The variability of bite force measurement between sessions, in different positions within the dental arch. J Oral Rehabil 1998; 25(9):681-686.

23. Johnsen SE, Svensson KG, Trulsson M. Forces applied by anterior and posterior teeth and roles of periodontal afferents during hold-and-split tasks in human subjects. Exp Brain Res 2007;178(2):126-134.

24. Neill DJ, Kydd WL, Nairn RI, Wilson J. Functional loading of the dentition during mastication. J Prosthet Dent 1989;62(2):218-228.

25. Yurkstas AA. The masticatory act: a review. J Prosthet Dent 1965;15:248-262.

26. Soderling E. Practical aspects of salivary analyses. In: Tenovuo JO, editor. Human Saliva: Clinical Chemistry and Microbiology. Boca Raton: CRC Press 1989:6-19.

27. Staninec M, Kim P, Marshall GW, Ritchie RO, Marshall SJ. Fatigue of dentin-composite interfaces with four-point bend. Dent Mater 2008;24(6):799-803.