2014년 2월
석사학위 논문

# Effect of Desensitizing Agent and tooth brushing on Dentin Adhesion of a Two-step Self-etch Adhesive

조선대학교 대학원

치 의 학 과

최 한 솔

# Effect of Desensitizing Agent and tooth brushing on Dentin Adhesion of a Two-step Self-etch Adhesive

자가 부식형 접착제의 상아질 결합에
지각과민 처치제와 칫솔질이 미치는 영향

2014년 2월 25일

조선대학교 대학원

치 의 학 과

최 한 솔

# Effect of Desensitizing Agent and tooth brushing on Dentin Adhesion of a Two-step Self-etch Adhesive

지도교수 민 정 범

이 논문을 치의학 석사학위신청 논문으로 제출함.

2013년 12월

조선대학교 대학원

치 의 학 과

최 한 솔

최한솔의 석사학위논문을 인준함.

위원장　조선대학교　교수　김 진 수　인

위　원　조선대학교　교수　황 호 길　인

위　원　조선대학교　교수　민 정 범　인

2013년　12월

조선대학교 대학원

# CONTENTS

# TABLE LEGENDS

# FIGURE LEGENDS

# 국 문 초 록

## 자가 부식형 접착제의 상아질 결합에 지각과민 처치제와 칫솔질이 미치는 영향

최 한 솔
지도교수 민정범
조선대학교 대학원
치의학과

본 연구의 목적은 상아질 지각 과민 처치제 그리고 지각과민 처치제 적용 후 칫솔질이 two-step self-etch adhesive의 미세 인장 결합 강도에 미치는 영향을 조사하는 것이었다. 인간 제 3 대구치 24개를 사용하였으며, 4개는 SEM 분석 그리고 나머지 20개는 미세인장강도 분석에 사용되었다. 미세인장강도 시험을 위한 20개 치아는 편평한 상아질 면이 얻어지도록 법랑질을 제거하고 무작위로 4가지 실험군으로 분류하였다. 1군은 대조군으로서 아무런 전처리를 시행하지 않고 Clearfil SE primer and bond를 사용하여 접착제를 도포한 후 즉시 Filtek Z250 복합레진을 사용하여 수복하였다. 2군은 지각과민 처치제인 Seal&Protect를 처리한 후 1군과 같은 방법으로 접착제 처리 및 복합레진 수복을 시행하였다. 3군과 4군은 지각과민 처치제에 대한 칫솔질의 영향을 평가하기 위하여 설계되었는데, 지각과민 처치제를 처리한 후 3군은 5,000 cycle, 4군은 10,000 cycle의 칫솔질을 시행한 후 1군과 같은 방법으로 접착제 처리 및 복합레진 수복을 시행하였다. 이후 미세인장 결합강도를 측정하였다. 나머지 4개의 치아는 SEM 분석을 시행하였다. 실험 결과, 1군은 2군, 3군, 4군보다 유의적으로 높은 수준의 결합 강도를 보였다($P < 0.05$). 반면에, 2군, 3군, 4군 간에는 통계적으로 유의한 차이가 나타나지 않았다. SEM 분석 결과는 미세인장강도 실험 결과를 뒷받침하였다. 결론적으로, 상아질 지각 과민 처치제인 Seal&protect의 적용은 two-step self-etch adhesive의 상아질 결합 강도를 유의적으로 감소시켰다. Seal&protect 적용 후 10,000 cycle 이하의 칫솔질은 결합 강도에 유의적인 영향을 미치지 못하였다.

# I. Introduction

Dentin hypersensitivity is a very frequent clinical problem that affects about 15% of the population.[1] It is defined as a short, sharp pain in response to evaporative, tactile, thermal, or chemo-osmotic stimulation of exposed dentin in teeth where there is no existence of other dental defect or pathology.[2] This clinical problem may arise as a result of loss of enamel and/or exposure of underlying dentin on root surface.[3] And periodontal disease, periodontal treatment, and inappropriate tooth brushing habits can also result in gingival recession leading sensitive teeth.[4]

Currently the most widely accepted mechanism for dentin hypersensitivity is the Hydrodynamic Theory described by Brännström. It suggests that rapid movement of fluids within the dentinal tubules results in a deformation of nerve fibers wrapped around the odontoblast cells.[5] Therefore, materials that occlude dentin tubules to a certain extent can potentially decrease fluid filtration across the dentin and relieve sensitivity.[6,7] Among dozens of treatment modalities for management of sensitivity, resin-based desensitizing agents are used more recently. They are generally acidic resins which bond to dentin by producing a resin-dentin hybrid layer and occluding the dentin tubules with resin plugs.[8]

In some cases, dental clinicians may perform resin composite restorations after applying other desensitizing agents due to continuing sensitivity or progressed cervical abrasion. Desensitizing agents could affect the bond strength of an adhesive to dentin. Several studies have evaluated the effect of desensitizers on the bond strengths of adhesive restorations but It is controversial. [9,10,11]

Furthermore abrasion by daily tooth brushing may affect the restorations.[12] Thus, abrasion and loss of desensitizing agents as a dentinal sealer also have a clinical

significance. However, there are very few reports on the effect of abraded desensitizers on the bond strengths of adhesive restorations.

For this reason, aim of this study was to evaluate the effect of a pre-treatment with a dentin desensitizer and subsequent tooth brushing on the microtensile bond strength of two-step self-etch adhesive to dentin. The first null hypothesis of this study was that dentin desensitizer does not affect the bond strength of two-step self-etch adhesive to dentin. And the second null hypothesis was that tooth brushing after applying desensitizer does not affect the bond strength of two-step self-etch adhesive to dentin coated with desensitizer.

# II. Materials and Methods

## 1. Specimen preparation

The specimen preparation procedure is schematically illustrated in Figure 1. Twenty four, unrestored, non-carious human third molars were used within 1 month after extraction. The teeth were cleaned of periodontal tissue residue using a periodontal scaler and were stored in distilled water at room temperature. Storage water was changed daily. The occlusal enamel was removed by sectioning the crown perpendicular to the long axis of the tooth using a model trimmer (Se-ki, Seoul, Korea) under copious water lavage to achieve a flat superficial dentin surface. And dentin surface was wet-polished with abrasive paper (6000 grit; Silicon Carbide Water Proof Abrasive Paper Electro Coated, Daesung, Korea) with running tap water for 1 min to obtain a smooth dentin surface and rinsed for 1 min.

Twenty teeth were used for microtensile bond strength test and four teeth were used for SEM examination. For microtensile bond strength test, the teeth (N=20) were then randomly divided into four groups and each group has 5 specimens.

Group 1 served as a control. They were designed for evaluating microtensile bond strength of teeth does not affected by desensitizer. They were not pretreated and Clearfil SE Bond (Kuraray, Osaka, Japan) as dentin adhesive applied to dentin surfaces following the manufacturer's instructions. Spectrum 800 (Dentsply Caulk, Milford, DE. USA) with an output intensity of 400 mW/cm$^2$ was used for light curing. Then 4-mm-thick build-ups of composite (Filtek Z250; 3M ESPE, St. Paul, MN, USA) were placed, with increments limited to 1-mm. Each increment was light-cured for 40s. Then the specimens were stored in distilled water at 37℃ for 24 h.

In group 2, dentin surface was coated with dentin desensitizer, Seal&Protect (Dentsply Detrey, Konstanz, Germany) following the manufacturer's instructions. And dentin adhesive

and composite was applied in the same way as described for group 1. Composition and manufacturer of desensitizing agent, dentin adhesive and composite used in this study were shown in Table 1.

Group 3 and 4 were designed for evaluating the effect of tooth brushing on the desensitizing agent. After being treated with dentin desensitizer following the manufacturer's instructions, the specimens were subjected to the tooth brushing test. The test was performed in an automatic brushing machine (Tooth Brush Tester designed by department of preventive dentistry, College of dentistry, Chosun University, Gwangju, Korea) with toothpaste slurry (Figure 2). The brushing machine equipped with two toothbrush heads (27mm in length, 10mm in width, medium hardness, 10mm filament length) and it was adjusted to have reciprocating linear motion to the tooth brushes. Brushing frequency was 3 cycles per second and a brushing load was 300g. The toothpaste slurry was prepared by mixing toothpaste (2080 original alpha green, Aekyung, Seoul, Korea) and tap water at a 1:3 proportion. After each 10,000 brushing cycles, the slurry was renewed. The specimens were brushed with 5,000 brushing cycles for group 3 and 10,000 cycles for group 4. According to other report, 10,000 brushing cycles reflect about 1 year of toothbrushing.[13] Therefore, our test simulate in 6 months and 1 year of tooth brushing. After the whole procedure of brushing, each specimens were rinsed with running tap water for 1 min. Then group 3 and 4 was applied with dentin adhesive and composite in the same way as described for group 1.

**Table 1.** Desensitizer, adhesive, composite used in the study

| Brand | Material | Composition | Manufacturer |
|---|---|---|---|
| **Seal&Protect®** | Desensitizer | Methacrylate resins, amorphous silica, PENTA, nanofillers, butylated hydroxytoluene, cetylaminohydro-fluoride, triclosan, photoinitiators, acetone | Dentsply, Detrey, GmbH, Konstanz, Germany |
| **Clearfil™ SE Bond** | Two-step self-etch adhesive system | **Primer**; MDP, HEMA, hydrophilic dimethacrylate<br>**Bond**: MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, DL-camphorquinone, N,N-diethanol-p-toluidine and silanated colloidal silica | Kuraray, Osaka, Japan |
| **Filtek™ Z250** | composite | Bis-GMA, BisEMA, UDMA, TEGDMA, Silane treated ceramic | 3M ESPE, St. Paul, MN, USA |

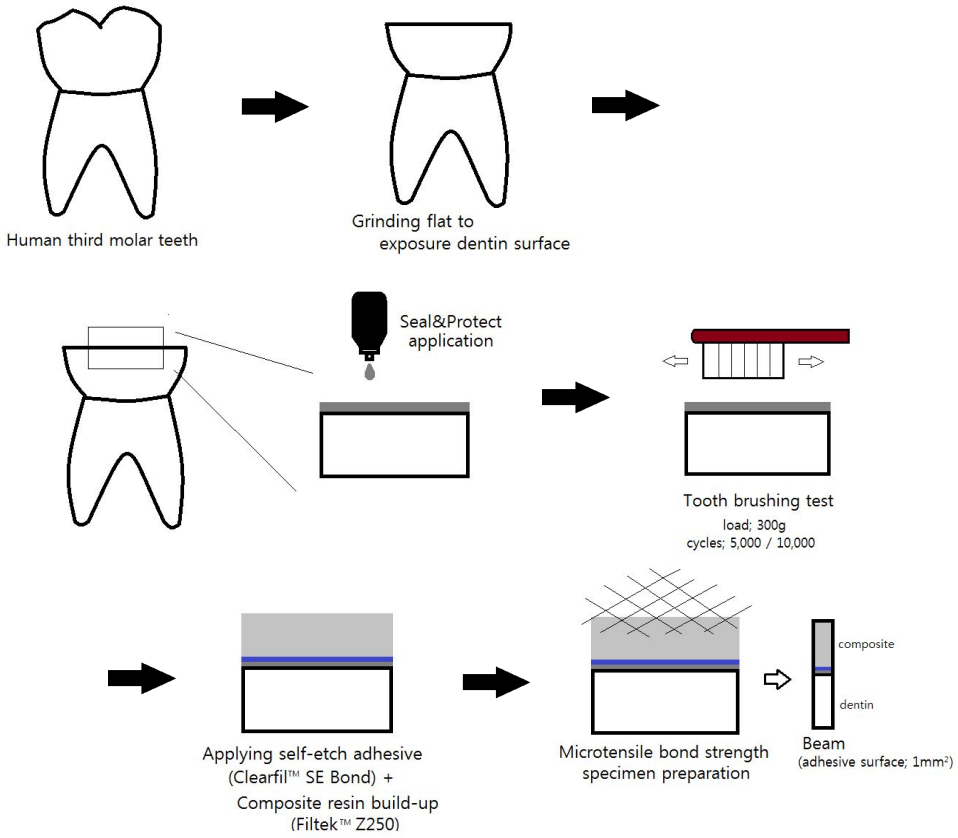


**Figure 1.** Schematic representation of the specimen preparation.


**Figure 2.** Automatic brushing machine (Tooth Brush Tester)

## 2. Microtensile bond strength test

After being stored in distilled water at 37℃ for 24 h, the specimens were embedded in acrylic blocks using sticky wax (Kerr corporation, Orange, CA, USA). Then they were placed on a low-speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) to produce 1mm X 1mm adhesive surface area beams under water cooling. Each beam consists of composite resin and dentin. The dimension of each beam was measured using digital calipers and the bonded area was calculated for subsequent conversion of microtensile strength values into units of stress (MPa).

The beams were attached with cyanoacrylate adhesive (Zapit; DVA, Corona, CA, USA) to a testing apparatus and tensile load was applied with a microtensile tester (Micro Tensile Tester; Bisco, USA) at a cross-head speed of 0.5mm/min, until fracture (Figure 3).

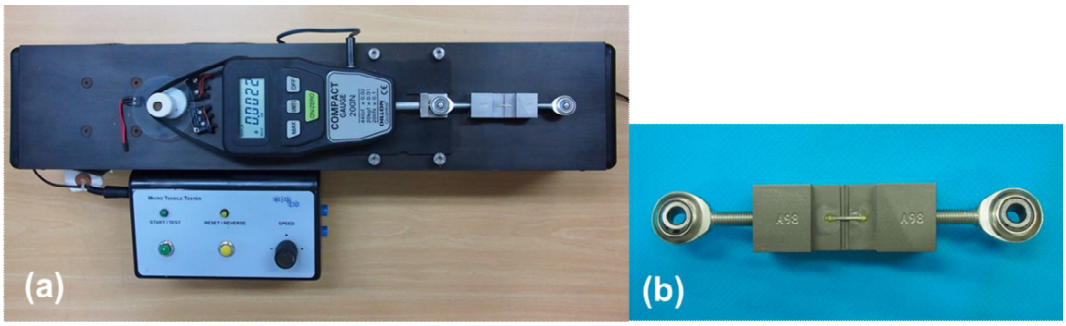


**Figure 3.** (a) Microtensile tester; (b) Beam attached on microtensile device.

## 3. Statistical analysis

Results were expressed in MPa and the data was submitted to a one-way analysis of variance (ANOVA) and Tukey's test using SPSS™ Ver 12.0 (SPSS Inc, Chicago, IL, USA). *P* values of 0.05 were considered to indicate statistical significance.

## 4. SEM examination

For SEM examination, one tooth of each group was prepared. The teeth were treated in the same way as described above. Then they are thoroughly rinsed and air-dried for 24 h. For dehydration, specimens were then placed in an incubator (HB-502; Han-Baek, Korea, setting temperature of 37℃) for 1 week.

The specimens were mounted on SEM stubs, sputter-coated with gold-palladium and examined in a SEM (S-4800; Hitachi, Tokyo, Japan). Photographs of the most expressive regions were taken at x3,000 magnification.

# III. Results

## 1. Microtensile bond strength

Mean microtensile bond strength values and significant differences among the groups are shown in Table 2 and Figure 4. Mean microtensile bond strength of group 1 which had no treatment showed a significantly higher bond strength than group 2 (desensitizer only), group 3 (desensitizer and 5,000 cycles of brushing) and group 4 (desensitizer and 10,000 cycles of brushing) ($p < 0.05$). There was no significant difference was found among group 2, 3 and 4 ($p > 0.05$).

**Table 2.** Mean and SD values for microtensile bond strength (MPa)

| Group | Number | Mean | S.D. |
|---|---|---|---|
| **1 (Control)** | 30 | 38.66 [a] | 15.27 |
| **2** | 30 | 29.95 [b] | 6.12 |
| **3** | 30 | 30.38 [b] | 11.59 |
| **4** | 30 | 31.10 [b] | 6.40 |

* Values marked by different superscript letters are significantly different. ($p<0.05$)

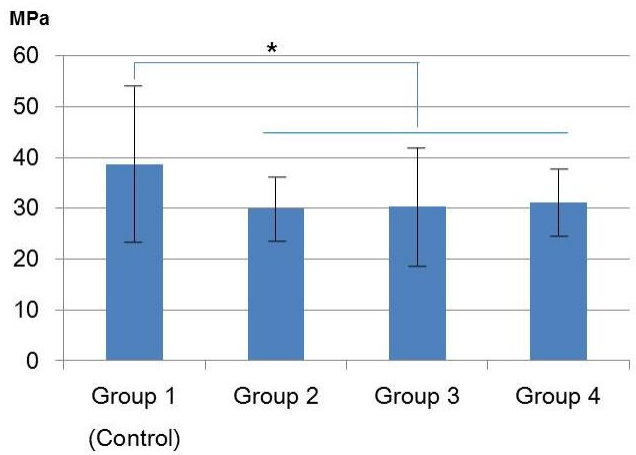


**Figure 4.** Bar diagram showing mean values for microtensile bond strength (*$p < 0.05$).

## 2. SEM analysis

Fig. 5 shows the microstructure alterations of the dentin surface after treatment with desensitizer and tooth brushing test. In group 1, exposed dentin tubules were observed (Figure 5a). In group 2 which was coated with desensitizer, dentin tubules were completely occluded with resin-based desensitizing material (Figure 5b). In group 3 which was coated with desensitizer and brushed for 5,000 cycles, resin-based material covering dentinal tubule was abraded to some degree and some areas were free of resin (Figure 5c). Group 4 which was coated with desensitizer and brushed for 10,000 cycles is similar with group 3. But, resin-based material covering dentinal tubule was more abraded and had smoother surface than those of group 3 (Figure 5d).

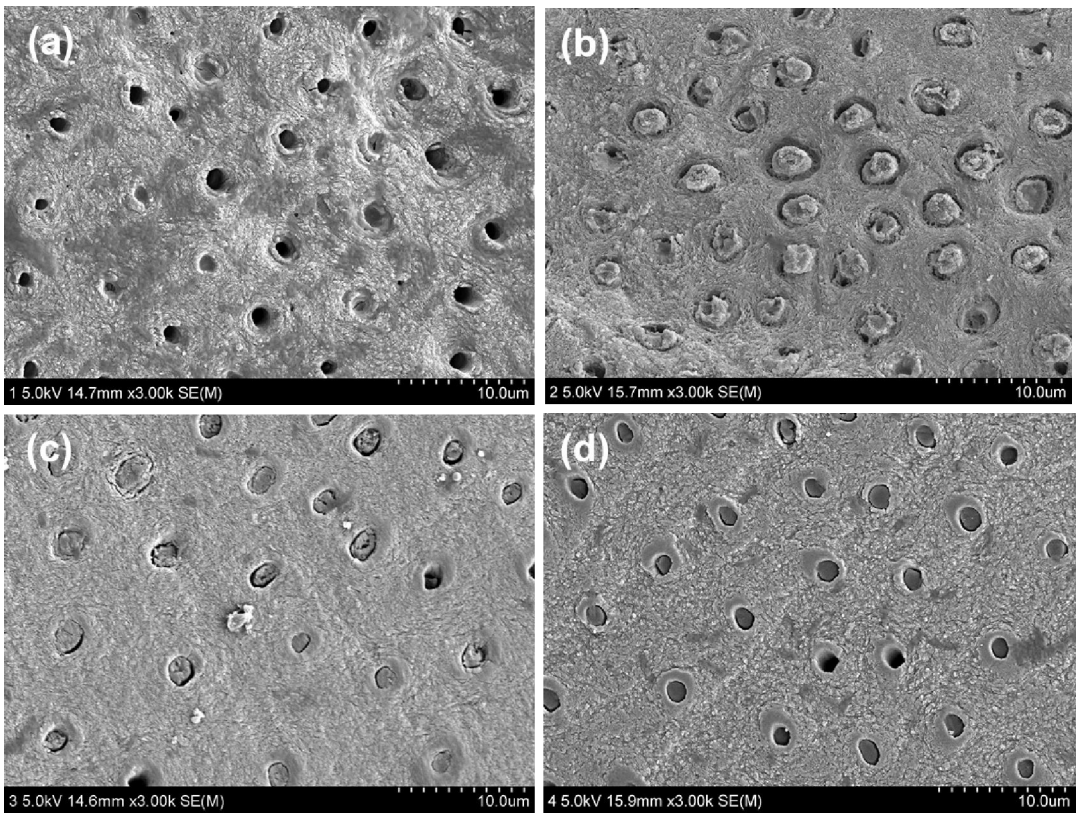


**Figure 5.** SEM view of the dentin surface after treatment with the desensitizer and tooth brushing test. (a) Group 1; (b) Group 2; (b) Group 3; (d) Group 4. x3,000 magnification.

# IV. Discussion

The aim of the present study was to investigate the effect of dentin desensitizer on the bond strength of two-step self-etch adhesive to dentin using twenty human third molars. Seal&Protect was used as desensitizing agent in this study. It is a resin-based material that does not contain 2-hydroxyethyl methacrylate (HEMA) and specially designed to seal the open dentin tubules in hypersensitive teeth. It is a self-adhesive, light curing, translucent sealing material and contains two important components; antimicrobial agent triclosan and the nanofiller. Numerous studies have described the impact of many desensitizers on dentin adhesives[9,10,11], but, there are few studies using Seal&Protect and there is no study investigating the effect of brushing on the bond strength of adhesive to dentin coated with Seal&Protect.

Dentin bonding is based on the formation of a resin-infiltrated layer in the conditioned intertubular and peritubular dentin. After polymerization, resin monomers may form a micro-mechanical bond with the primed dentin, so-called hybrid layer. It is the principle mechanism of bonding.[14] Thus, to achieve a satisfactory dentin adhesion, the open tubules and exposed collagen-rich meshwork should be infiltrated by resin monomers entirely and homogeneously. However, Seal&Protect reduced infiltration of resin monomer of dentin adhesive by occluding dentin tubules in this study. Therefore, weak hybrid layer was formed and microtensile bond strength was significantly decreased.

Additional tooth brushing test leading abrasion of Seal&Protect occluding dentinal tubules was performed in this study. Automatic brushing machine is used to have an equal effect. According to the previous clinical studies, the mean brushing force values applied by subjects were 267±73 g[15] and 330±109 g[16]. Thus the 300 g vertical load was used as the tooth brushing force in this study. However, brushing method and brushing force may

differ from individual to individual in reality. Therefore, the effect of tooth brushing could vary.

In this study, the microtensile bond strength increases as brushing cycles increase but there was no significant difference. Even after 10,000 cycles of brushing, bond strength was significantly lower than control group. These results are similar with the SEM view. After 5,000 cycles of brushing, resin-based material was loss partially and some dentin tubules were not covered but, most of the area was still occluded with the material. After 10,000 cycles of brushing, SEM view was similar. These results may be explained by composition of Seal&Protect, in particular nanofiller. Generally, in the resin-based materials containing additional filler particles, the filler particles protect the softer resin-matrix from abrasion.[17] According to a recent study, desensitizing material containing glass fillers show higher toothbrush wear resistance.[18] This result reflect the role of filler. For this reason, Additional procedure would be recommended like grinding dentin surfaces with burs before applying dentin adhesive. Tooth brushing more than 10,000 cycles may also change the result. Thus, further study is necessary to obtain acceptable bond strength of adhesive to dentin that applied with desensitizing agents.

This in vitro study was done with extracted teeth without simulating dentinal fluid pressure, so it is difficult to compare the results with clinical conditions. And another limitation of this study is that two-step self-etch dentin adhesive was used only. In future studies, it would be advantageous to use different dentin adhesives especially total-etch adhesive.

# V. Conclusion

This study evaluated the effect of pre-treatment with a dentin desensitizer and subsequent tooth brushing on the microtensile bond strength of two-step self-etch adhesive to dentin. Within the limitations of this study, it is concluded that pretreatment of desensitizing agent (Seal&Protect) reduced the bond strength of two-step self-etch adhesive to dentin and tooth brushing up to 10,000 cycles after applying desensitizing agent does not affect the bond strength of adhesive to dentin coated with desensitizer.

Thus, first null hypothesis was rejected as desensitizer affected the bond strength to dentin. On the other hand, the study failed to reject the second null hypothesis as tooth brushing after applying desensitizer does not affect the bond strength to dentin coated with desensitizer.

# References


1. Addy M. Clinical aspects of dentin hypersensitivity. *Proc Finn Dent Soc* 1992;88(Suppl 1):23-30.
2. Addy M, Urquart E. Dentine hypersensitivity: Its prevalence, aethiology and clinical management. *Dental Update* 1992;19:407-408.
3. Dababneh RH, Khouri AT, Addy M. Dentine hypersensitivity—an enigma? A review of terminology, epidemiology, mechanisms, aethiology and management. *Br Dent J* 1999,187:11:606-611.
4. Walters PA. Dentinal hypersensitivity: A review. *J Contemp Dent Pract* 2005;6:107-117.
5. BrännströmM, Aström A. The hydrodynamics of the dentine; its possible relationship to dental pain. *Int Dent J* 1972;22:219-227.
6. Jain P, Vargas MA, Denehy GE, Boyer DB. Dentin desensitizing agents: SEM and X-ray microanalysis assessment. *Am J Dent* 1997;10:21-26.
7. Pashley DH, Livingston MJ, Greenhill JD. Regional resistances to fluid flow in human dentine in vitro. *Arch Oral Biol* 1978;23:807-810.
8. Camps J, About I, Van Meerbeek B, Franquin JC. Efficiency and cytotoxicity of resin-based desensitizing agents. *Am J Dent* 2002;15:300-304.
9. Arisu HD, Dalkihç E, Üçtaşli MB. Effect of desensitizing agents on the microtensile bond strength of a two-step self-etch adhesive to dentin. *Oper Dent* 2011;36:153-161.
10. Pei D, Liu S, Huang C, Du X, Yang H, Wang Y, Deng D. Effect of pretreatment with calcium-containing desensitizer on the dentine bonding of mild self-etch adhesives. *Eur J Oral Sci* 2013;121:204-210.
11. Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI. Integrating oxalate desensitizers with total-etch two-step adhesive. *J Dent Res* 2003;82:703-707.
12. Attin T, Buchalla W, Hellwig E. Effect of topical fluoride application on toothbrushing abrasion of resin composites. *Dent Mater* 2006;22:308-313.
13 . Goldstein GR, Lerner T. The effect of toothbrushing on a hybrid composite resin. *J Prosthet Dent* 1991;66:498-500.
14. Nakabayashi N., Dentinal bonding mechanisms. *Quintessence Int* 1991;22:73-74.

15. van der Weijden GA, Timmerman MF, Reijerse E, Snoek CM, van der Velden U. Toothbrushing force in relation to plaque removal. *J Clin Periodontol* 1996;23:724-729.
16. Van der Weijden GA, Timmerman MF, Danser MM, Van der Velden U. Relationship between the plaque removal efficacy of a manual toothbrush and brushing force. *J Clin Periodontol* 1998;25:413-416.
17. Bayne SC, Taylor DF, Heymann HO. Protection hypothesis for composite wear. *Dent Mater* 1992;8:305-309.
18. Gando I, Ariyoshi M, Ikeda M, Sadr A, Nikaido T, Tagami J. Resistance of dentin coating materials against abrasion by toothbrush. *Dent Mater J* 2013;32:68-74.