Chiang Mai Dental Journal

Original Article

The Effect of Surface Treatments of Exposed Dentin on Dentin Permeability and Shear Bond Strength of Resin Cement under Simulated Pulpal Pressure Condition

Napatsorn Dusadeedumkoeng[1], Pattaranat Banthitkhunanon[2], Sitthichai Wanachantararak[3]
[1]Graduate student, Department of Prosthodontics, Faculty of Dentistry, Chiang Mai University, Thailand
[2]Department of Prosthodontics, Faculty of Dentistry, Chiang Mai University, Thailand
[3]Department of Diagnostic Sciences and Oral Biology, Faculty of Dentistry, Chiang Mai University, Thailand

Received: April 14,2021 • Revised: June 1, 2021 • Accepted: July 20, 2021

Corresponding Author: ***Assistant Professor Pattaranat Banthitkhunanon****, Department of Prosthodontics, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand. (E-mail:* ***pat.dentcm@gmail.com****)*

Abstract

Objective: The objective of this study was to evaluate the effect of surface treatments application of exposed dentin with Teethmate® (TDA) and Portland cement on dentin permeability and shear bond strength of resin cement under simulated pulpal pressure conditions in extracted human teeth.

Materials and Methods: Sixty extracted teeth were divided equally into six groups; control, TDA and Portland cement groups with and without simulated 15 cmH_2O pulpal pressure. Each surface treatment was randomly applied to dentin surface. Dentin permeability was evaluated for simulated pulpal pressure groups by recorded fluid droplets on dentin surface using replica technique. The replica was examined under scanning electron micrograph. The specimen was re-polished and re-applied with the same surface treatment. The composite rod was bonded to dentin with self-etched resin cement, and the shear bond strength was tested. The data were analyzed using Two-way ANOVA and Tukey's multiple comparisons.

Results: Specimens with simulated pulpal pressure had significantly lower shear bond strength than without pulpal pressure ($p<0.01$). Surface treatment groups, TDA and Portland cement, showed significantly higher shear bond strength than the control group under simulated pulpal pressure condition ($p<0.05$), while no significant difference was seen in non-simulated pulpal pressure groups. Scanning electron micrograph showed that both surface treatment groups had significantly less permeability of dentin as smaller fluid droplets were recorded.

Conclusions: Dentin surface treatments effectively reduced dentin permeability and increased shear bond strength of resin cement in simulated pulpal pressure conditions. But there were no advantages over the control group for non-simulated pulpal pressure condition.

Keywords: bond strength, dentin permeability, Desensitizer, Portland cement, resin cement

Introduction

After dentin was exposed, the vital tooth has a spontaneous outward flow of dentinal fluid due to 15 cmH_2O pulpal tissue fluid pressure.[1] The flow acts as a self-defense mechanism against the penetration of bacterial and their toxins which also interfere with the penetration of dental adhesive into demineralized dentin causing the formation of an incomplete hybrid layer[2,3], which weakens the bond strength. The new generation of dental adhesive could affect and have a lower bond strength of resin cement in vital pulp or simulated pulpal pressure teeth when compared with non-vital or extracted tooth.[4] Removing a smear layer with acid treatment during bonding process may also increase dentin permeability and decreased shear bond strength[5], as the presence of a smear layer might reduce permeability and improve shear bond strength.

In order to limit dentin permeability, several forms of calcium phosphate,[6] potassium oxalate[7] and calcium silicate[8] has been used as surface treatment to seal dentinal tubules.[9,10] After application, they produced non-soluble crystal occluded dentinal tubules 5 μm deep for TDA[11] and 4-6 μm for Portland cement.[12] Both materials are effective on occluding dentinal tubules by inducing hydroxyapatite crystal formation and reduce dentin permeability.[11,13]

Emerging fluid droplets on dentin surface as a result of dentin permeability can be monitored by using the impression and replica technique first used to record sweat droplets in rat paws by Bharali *et al.*[14], which was later adopted to monitor fluid droplets on dentin surface in permanent and deciduous human teeth.[15-17] This technique is convenient and useful for monitoring the surface of dentin before and after surface treatment without altering the surface. However, the size and shape of fluid droplets obtained from this technique were virtually dependent on the impression material used. The scanning electron micrograph showed larger droplets when recorded using Xantopren VL plus® (Heraeus, Kulzer, Germany[15,16] while finer droplets were reported when using other impression material.[17,18] This technique can be used to monitor the fluid droplet on dentin precisely. Little is known about the effects of dentin surface treatment on dentin permeability and the shear bond strength of resin cement. The objective of this study was to evaluate the effect of surface treatments application of exposed dentin with TDA (Kuraray Noritake Dental, Tokyo, Japan) and Portland cement (Tiger, The Siam Cement Pcl, Bangkok, Thailand) on dentin permeability and shear bond strength of resin cement under simulated pulpal pressure condition in extracted human teeth.

Materials and Methods

Sixty intact extracted human maxillary third molars from 16-40 years old patients were included in this study. Immediately after extraction, the teeth were stored in an aqueous solution of 1% chloramine-T for 1 week, then stored in grade 3 distilled water at 4°C until used within 6 months. The use of human tissue was approved by The Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand. (Certificate of ethical clearance No.15/2020).

The roots were cut off at 2 mm apically to the cemento-enamel junction. The remaining pulpal tissue was removed with barbed broach under water to prevent trapping air bubbles inside the pulp chamber. The cut surface was attached to an acrylic sheet (3 mmx3 mm with 2 mm diameter hole) with cyanoacrylate glue. A conical acrylonitrile butadiene styrene (ABS) plastic tube (O.D.=2 mm and i.d.=1.2 mm) was adhered to the hole of acrylic sheet with cyanoacrylate glue. The whole specimen was embedded in epoxy resin in a polyvinylchloride (PVC) ring with the longitudinal axis of the tooth position parallel to the horizontal plane (Figure.1). After complete polymerization, the pulp chambers of simulated pulpal pressure groups were filled with 0.9% NSS and connected to a manometer. All specimens were stored under distilled water at 37°C for 24 hours until used.

The enamel at buccal surface was removed with a low-speed precision cutting machine (Isomet™ 1000 precision saw, Buehler, U.S.A) until exposed dentin, and another removed another 1 mm of dentin off. The cut surface was polished using 400-grit silicon carbide paper under running water for 10 seconds. For the simulated pulpal pressure groups, 15 cmH_2O hydrostatic pressure was carefully connected to the pulp chamber of specimen via plastic connector, as to avoid trapping air bubbles in the system.

Sixty samples were equally and randomly divided into 6 groups in order to test two groups of surface treatment agents, TDA, Portland cement and control groups with and without simulated pulpal pressure. TDA, con-

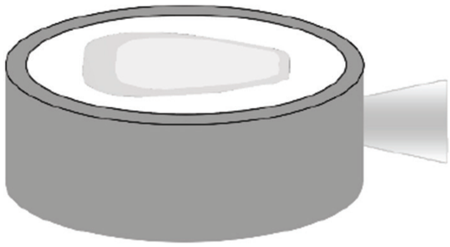

Figure 1: Schematic diagram demonstrates the preparation of tooth specimen embedded in epoxy resin within a PVC tube, and a plastic tube connected to the pressure chamber. The dentine at buccal surface is exposed for monitoring dentin permeability and for shear bond strength test.

taining a mixture of tetracalcium phosphate and anhydrous dicalcium phosphate, was used as representative of calcium phosphate. Portland cement which composed of tricalcium silicate and dicalcium silicate is a wildly available product in construction supply stores.

The specimens were separated as follows: Group A=Control without simulated pulpal pressure, Group B=TDA without simulated pulpal pressure, Group C= Portland cement without simulated pulpal pressure, Group D=Control with simulated pulpal pressure, Group E=TDA with simulated pulpal pressure, and Group F=Portland with simulated pulpal pressure.

Two dentin surface treatment agents, TDA and Portland cement, were selected and randomly applied on dentin to reduce dentin permeability. According to the manufacture's instructions, the powder and liquid of TDA were mixed using the ratio accordingly within 30 seconds and applied on the dentin for 45 seconds with a microbrush. For Portland cement group, the mixture of a gram of powder and 1.5 ml of water was applied on dentin with a microbrush and left for 2.5 minute.(12) The excess surface treatment agent was removed using a water jet from a triple syringe before being stored in the humidity chamber at 37°C for 24 hours.

A condensation silicone (Xantopren VL plus®, Henry Schein, Inc., Northen Ireland, UK) was used to take impression of the dentin surface of specimens in the groups with simulated pulpal pressure as it has more hydrophobicity. The dentin surface was carefully dried and left for 30 seconds. A small volume of impression material mixture was gently flowed on dentin surface and left for 5 minutes allowing it to completely set, while care was taken to prevent fluid droplets being compressed by the weight of material. The polyether impression material (Impregum; 3M ESPE, St Paul, MN, USA) was then poured into the previous impression to make a replica. After the impression was completely set, it was processed for examination under a scanning electron microscope (JSM-5910LV, JEOL, Massachusetts, USA).

In order to validate the impression and replica technique, the fluid droplets on dentin surface, imprint on an impression and replica polyether were observed and recorded in video format under a stereomicroscope with x15 magnification. Optical images of those were taken with Canon EOS RP using a macro reverse adapter ring and 18-55 mm lens.

The SEM image was taken at mid-buccal surface area at 500x magnification with 1280x960 pixels resolution as TIFF format. The droplet number and area were calculated using particle analysis function of ImageJ for window V.1.52 (National Institutes of Health, Bethesda, MD). In brief, the SEM images were assessed using threshold function that separated droplets from the background, followed by particle analysis function to calculate both area and number of droplets.(18)

After the impression was taken, the same surface of specimens of simulated pulpal pressure groups were re-polished with 400-grit silicon carbide paper for 2 seconds in order to renew the cutting surface and get rid it of any contamination caused by an impression. The same surface treatment agent was replied and the specimen was kept in distilled water for 24 hours. After storage, all specimens were cleaned with pumice slurry water for 5 seconds, washed for 20 seconds with water jet from triple syringe. The area of bonding was limited by an adhesive tape with 3 mm hole attached to the prepared dentin surface.

To test shear bond strength test (SBS), the composite resin rod (3 mm in diameter and 3 mm in height) was bonded to the dentin at the hole of adhesive type. The rod was made by filling a light-cured resin composite (Filtek™ Z350 XT, 3M ESPE, Seefeld, Germany) in the mold. The bonding surface was treated with an airborne-particle abrasive of 50 μm aluminum oxide under 35 PSI pressure for 15 seconds and cleaned in an ultrasonic cleaner machine (Easyclean, Renfert GmbH, Hilzingen, Germany) for 10 seconds. A mixture of resin cement (Panavia F2.0, Kuraray Medical Inc., Tokyo, Japan) was applied on the prepared surface of the rod and positioned with adhesive tape in the hole to bond with prepared dentin under a constant weight of 10 N for 10 seconds. The curing light from a light-curing unit (BlueLight Analytics™, 3M Deutschland, GmbH 41453 Neuss-Germany) with radiances of 550-650 mW/cm^2 was applied to the bonding interface for 40 seconds. The adhesive tape was removed from the dentin surface by using a blade to cut apart and peel off without pulling the rod. All specimens were kept in distilled water for 24 hours until SBS test.

A universal testing machine (Instron®, Instron Limited, Massachusetts, USA) with 500 N load cells and a cross head speed of 0.5 mm/minutes was used to test shear bond strength of all specimens. A knife-edge shear blade was positioned at an interface between dentin and the resin composite rod. SBS in megapascals (MPa) was calculated by divided force (N) with bonding area (mm^2) following the formula according to ISO/TS 11405:2003.

$$SBS = \frac{\text{Force (N)}}{Area\ \text{(mm2)}}\ \text{MPa}$$

The bond strength data of surface treatment groups and the present/absence of simulated pulpal pressure were analyzed with Two-way ANOVA and Tukey's multiple comparisons to examine the differences among groups (α=0.05). One-way ANOVA was used to compare the number and area of fluid droplets among surface treatment group.

Result

Fluid droplets were discovered on the surface of replica from all three simulated pulpal pressure groups (Figure 2).

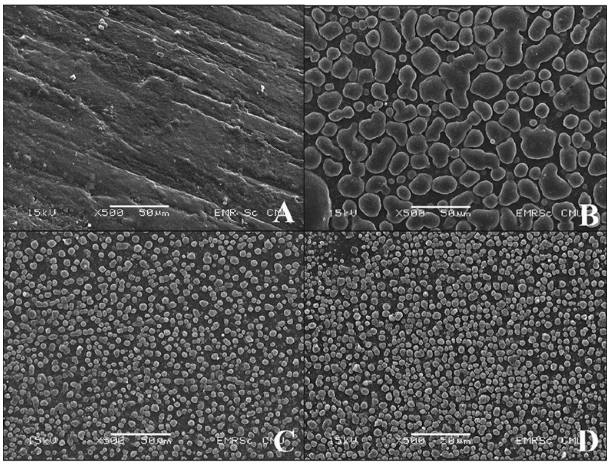

Figure 2: Representative scanning electron micrograph of dentin surface under simulated pulpal pressure (A) Control with -10 cmH_2O (B) Control with 15 cmH_2O (C) TDA with 15 cmH_2O (D) Portland cement with 15 cmH_2O.

In control group, droplets were significantly larger in diameter (23.26±11.14 μm) but significantly less in number (70.20±16.00 droplets) with irregular or elliptical shapes while the droplets on replica from TDA and Portland cement group were smaller in diameter (5.83± 3.68 μm and 5.97±3.20 μm), more similar and uniformly round in shape and greater in number (538.50±177.50 and 668.47±189.59 of droplets respectively) (Table 1). These results indicate that fluid droplets in control resulted from the merging of small droplets to form larger droplets. The individual droplet was seen in the replicas from both surface treatment groups as the permeability was reduced.

Table 1: Mean fluid area of droplet and number of droplets with standard deviation calculated with a standardized area of 45,800 μm^2.

Group	Mean±SD		
	Diameter of individual droplet (μm)	Total fluid area (%)	Number of droplet
Control	23.26 ±11.14*	65.88 ±7.79*	70.20±16.00*
TDA	5.83 ±3.68	49.99 ±7.47	538.50±177.50
Portland cement	5.97 ± 3.20	47.80 ±4.80	668.47±189.59

* Group identified with an asterisks are significantly different from other groups (p<0.05)

The emerging fluid on dentin was proved with a high magnification camera. The pictures of fluid droplets on dentin surface, negative imprint of impression and replica polyether are presented in Figure 3.

Within 48,500 μm^2 area in one SEM image, the percentage of fluid area for control group was 65.88% ±7.79%, significantly greater than those of TDA and Portland cement groups which were 49.98%±7.47% and 47.80% ±4.80% respectively (Table 1). The surface treatment reduced the dentin permeability by approximately 24.14% and 27.44%.

There were no significant differences in shear bond strength among control (15.54±1.67 MPa), Portland (15.14±2.11 MPa) and TDA (14.99±3.90 MPa) groups under absent pulpal pressure condition. But when applied simulated pulpal pressure, the shear bond strength was reduced significantly in all groups. However, TDA and Portland groups showed higher shear bond strength than control group under simulated pulpal pressure condi-

Figure 3: A sequence of images demonstrates impression and replica technique taken by digital camera at x15 magnification. (A) Optical image of a fluid droplet seen on dentin surface under simulated pulpal pressure condition after being mopped dry and leave it for 30 seconds. (B) Negative imprint of droplets discovered on the impression material taken from the same dentin. (C) Polyether replica demonstrates a similar pattern of fluid droplet recorded compare to fluid droplet on the dentin surface.

tion (8.65±2.10 MPa, 8.43±2.90 MPa, 5.52±2.64 MPa), respectively (Table 2).

Table 2: Mean shear bond strength values and standard deviation in MPa between dentin surface and resin cement. The shear bond strength of all groups was reduced significantly when applied simulated pulpal pressure.

Pulpal pressure	Mean±SD of shear bond strength (MPa)		
	Control	Calcium phosphate	Portland cement
No pulpal pressure	15.54±1.67	14.99±3.90	15.14±2.11
Simulated pulpal pressure	5.52±2.64*	8.65±2.10	8.43±2.90

* Group identified with an asterisks are significantly different from other groups within the same pulpal condition ($p<0.05$)

Pearson correlation analysis suggested that the shear bond strength value and area of fluid droplet in simulated pulpal pressure groups had a negative correlation ($p<0.05$) where smaller fluid area in TDA and Portland groups related to a higher shear bond strength of resin cement. (Figure 4) Failure mode in no pulpal pressure simulation groups showed a greater number of mixed failure than adhesive failure. These two failure modes were comparable in pulpal pressure simulation condition. However, no cohesive failure was found in this experiment.

Discussion

The fluid droplets were observed only under simulated pulpal pressure condition on the unetched dentin surface with smear layer presented.[15] However, when the pulpal pressure was set to -10cmH_2O, fluid droplets disappeared from the surface as the fluid flowed back into the dentinal tubules, corresponding with the dentin permeability studies using Evan's blue dye by Vongsavan and Matthews 1992.[1]

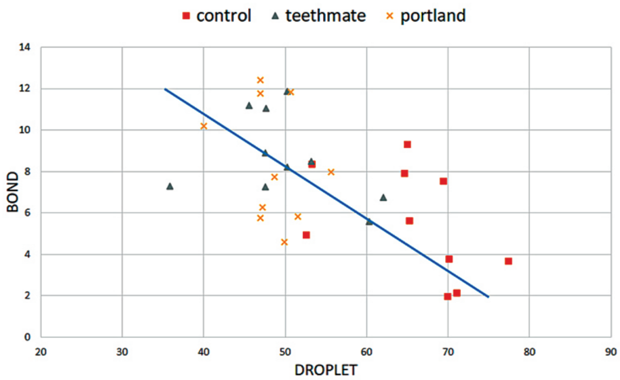

Figure 4: Correlation analysis between total fluid area of water droplets and shear bond strength. A significant ($p<0.05$) negative correlation is identified between the two variables.

The technique of recording fluid emerging on dentin surface by impression and replica technique used in previous studies[15,16] was proved by using a high magnification digital camera to take still images of fluid droplets on dentin surface, negative imprint in the impression and the replica. The video was recorded using the same camera to follow the pattern of emerging fluid droplets from a control group that showed small fluid droplets oozing from the dentin surface before joining together to form larger irregular droplets with time.

In this study, the diameter of recorded fluid droplets ranged from 5 to 24 μm, similar to previous studies.[15,16] In contrast, Sauro *et al.,* found smaller diameter (1-2 μm in diameter) and fewer droplets when using President® Impression material (Coltene AG, Altstatten, Switzerland.[17]

The differences in impression material may yield different results, according to Boening *et al,* Xantopren VL plus had the highest water contact angle (approximately 108°)[(19)] making it more hydrophobic than other impression materials which might have disadvantages in clinical use, but is advantageous and suitable for recording the fluid droplets in experiments because it has very low viscosity.

The total percentage of fluid area of both TDA and Portland cement groups indicated that the partially occluded dentinal tubules retarded the outward flow of dentinal fluid and significantly reduced dentin permeability when compared with control group. This coincided with the study by Sahin who found that sealing the dentinal tubule by adhesive bonding to reduce permeability of dentinal fluid increased effectiveness of the dental adhesive.[(18)] The present study discovered that the permeability of dentin after TDA and Portland cement treatment decreased significantly by 24.14% and 27.44% consecutively. These were less than the studies by Ishihata *et al.*[(20)] and Gandolfi *et al.*[(8)], which were 30-50% and 53% reduction. However, these surface treatment materials had less effectiveness in occluded dentinal tubules when compared with dental adhesive.[(18)] Sahin and colleagues found that the dentin permeability was reduced 61.35% with Gluma treatment and 82.52% with G bond treatment. Sauro *et al.,* showed that the Clearfil protect bond treatment lowered the permeability by 88.80%.[(17)]

Under pulpal pressure condition, as in vital tooth or simulated pulpal pressure, the shear bond strength of resin cement was significantly reduced[(4,21)] compared with control which was non-vital or un-simulated pulpal pressure. Corresponding with Alexandre and colleagues study,[(4)] this study found that the shear bond strength of the control group with simulated pulpal pressure was three times lower than that without pulpal pressure. Similar to vital tooth, polymerization of resin cement was impaired by the outward flow of dentinal fluid which could have resulted in failure of restoration and reduced retention of fixed prosthesis.[(21)] Moreover, the acidic monomer of primer in resin cement dissolved the occluded smear layer from the dentin surface and allowed dentinal fluid to flow out. This interfered with the infiltration of monomer into the decalcified matrix[(22,23)], which resulted in weakening shear bond strength.[(24,25)]

Surface treatment with TDA and Portland cement improved the bond strength in simulated pulpal pressure condition by decreasing the moisture on the dentin surface as seen in the SEM images, which corresponds with Pashley *et al*. study.[(25)] This provides evidence to support the idea that occlusion of dentinal tubule with non-soluble material could help to improve the bond strength of dental adhesive. As Hiraishi and colleagues mentioned earlier that adequate water is necessary for dentin bonding in terms of improving bond strength, but excess water on dentin surface will dilute adhesive monomer and reduce monomer infiltration, resulting in lower bond strength of adhesive.[(26)] On the other hand, Uğur and colleagues suggested that the application of TDA trended to improve bond strength by sealing dentinal tubules[(27)], but not significantly from their control group. Without simulated pulpal pressure, the shear bond strength of TDA and Portland cement groups showed no significant difference to control group, which is similar to other *in vitro* studies[(28,29)] that indicate no advantage on using surface treatment on non-vital tooth.

TDA and Portland cement materials reduced dentin permeability by occluded dentinal tubules up to 4-6 μm depth by deposition of hydroxyl apatite and calcium silicate crystals respectively.[(8,30)] The EDS study confirmed that calcium element on the surface of dentin increased after surface treatment with both materials.[(8,31)] This study provides evidence to support that these two surface treatment materials could be used as the permeability reduction to reduce outward flow of dentinal fluid and promote inward diffusion of resin monomer into decalcified matrix of dentin forming hybrid layer in presence of pulpal pressure as vital tooth.[(22,23)]

Mixed failure was more predominant than adhesive failure in all groups without simulated pulpal pressure, but they were comparable under simulated pulpal pressure condition. In contrast to other surface treatments using dental adhesive[(32)] and oxalate[(33)], an adhesive failure was mainly found between hybrid layer and luting cement. This provides evidence that these two surface treatment materials did not disturb the shear bond strength of dental adhesive, instead they seem to improve the adhesion of resin cement.

Even though this study used both TDA, a commercial product for hypersensitivity dentin treatment[(27,29,30)], and industrial-grade Portland cement[(8,13)], Portland cement could be developed further for use as dental material. Komabayashi *et al*. found that Portland cement has a majority of small 0.5-3 μm[34] particles, about 88% and 3-10 μm. While the average diameter of human dentinal tubule was 2.46±0.07[35] to 2.65±0.19[36] μm from superficial dentin to middle layer. The larger particles of Portland cement might not be able to penetrate into dentinal tubule. As the Portland cement can occlude dentinal tubule in the moisture condition, it is possible that Portland cement migth be an alternative material for use in permeability reduction and dentin sensitivity treatment effectively if it is investigated in more detail and developed for dental use. This research also provides evidence as a starting point for the future study and clinical use of dentin occlusion to improve bond strength in vital tooth.

Conclusions

TDA and Portland cements as a dentin surface treatment were effective for reducing dentin permeability and increasing shear bond strength of dental adhesive in simulated pulpal pressure condition representing vital tooth. There were no advantages over the control group under non-pulpal pressure condition or non-vital tooth.

Acknowledgments

This research is funded by Faculty of Dentistry, Chiang Mai University. The authors gratefully acknowledge Dr.Thanapat Sastraruji for his assistance in statistical analysis and Mr. Wisanu Boonsuk for SEM assistance.

Conflict of interest

The authors declare no potential conflict of interest and no competing interest in this study.

Reference

1. Vongsavan N, Matthews B. Fluid flow through cat dentine *in vivo*. Arch Oral Biol. 1992;37(3):175-85.
2. Hashimoto M, Ito S, Tay F, Svizero Nd, Sano H, Kaga M, *et al.* Fluid movement across the resin-dentin interface during and after bonding. J Dent Res. 2004;83:843-8.
3. Shafiei F, Memarpour M, Doozandeh M. Effect of oxalate desensitizer on the bonding durability of adhesive resin cements to dentin. J Prosthodont Res. 2012;56(3):187-93.
4. de Alexandre R, Santana V, Kasaz A, Arrais C, Rodrigues J, Reis AF. Effect of long-term simulated pulpal pressure on the bond strength and nanoleakage of resin-luting agents with different bonding strategies. Oper Dent. 2014;39(5):508-20.
5. Tao L, Pashley DH. The relationship between dentin bond strengths and dentin permeability. Dent Mater. 1989;5(2):133-9.
6. Geiger S, Matalon S, Blasbalg J, Tung M, Eichmiller FC. The clinical effect of amorphous calcium phosphate (ACP) on root surface hypersensitivity. Oper Dent. 2003;28(5):496-500.
7. Richardson D, Tao L, Pashley DH. Dentin permeability: effects of crown preparation. Int J Prosthodont. 1991;4(3):219-25.
8. Gandolfi MG, Silvia F, H PD, Gasparotto G, Carlo P. Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine. J Dent. 2008;36(8):565-78.
9. Lukomsky EH. Fluorine Therapy for exposed dentin and alveolar atrophy. J Dent Res. 1941;20(6):649-59.
10. Hoyt WH, Bibby BG. Use of sodium fluoride for desensitizing dentin. J Am Dent Assoc. 1943;30(17):1372-6.
11. Thanatvarakorn O, Nakashima S, Sadr A, Prasansuttiporn T, Ikeda M, Tagami J. *In vitro* evaluation of dentinal hydraulic conductance and tubule sealing by a novel calcium-phosphate desensitizer. J Biomed Mater Res B Appl Biomater. 2013;101(2):303-9.
12. Dong Z, Chang J, Deng Y, Joiner A. Tricalcium silicate induced mineralization for occlusion of dentinal tubules. Aust Dent J. 2011;56(2):175-80.
13. Gandolfi MG, Iacono F, Pirani C, Prati C. The use of calcium-silicate cements to reduce dentine permeability. Arch Oral Biol. 2012;57(8):1054-61.
14. Bharali LA, Burgess SA, Lisney SJ, Pearson D. Reinnervation of sweat glands in the rat hind paw following peripheral nerve injury. J Auton Nerv Syst. 1988;23(2):125-9.
15. Kerdvongbundit V, Thiradilok S, Vongsavan N, Matthews B. The use of the replica technique to record fluid emerging from exposed dentine. Arch Oral Biol. 2004;49:613-9.
16. Rangcharoen M, Sirimaharaj V, Wanachantararak S, Vongsavan N, Matthews B. Observations on fluid flow from exposed dentine in primary teeth: An *in vitro* study. Arch Oral Biol. 2017;83:312-6.
17. Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, *et al*. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater. 2007;23(6):705-13.
18. Sahin C, Cehreli Z, Yenigul M, Dayangac B. *In vitro* permeability of etch-and-rinse and self-etch adhesives used for immediate dentin sealing. Dent Mater J. 2012;31:401-8.
19. Boening KW, Walter MH, Schuette U. Clinical significance of surface activation of silicone impression materials. J Dent. 1998;26(5):447-52.
20. Ishihata H, Kanehira M, Finger W, Takahashi H, Tomita M, Sasaki K. Effect of two desensitizing agents on dentin permeability *in vitro*. J Appl Oral Sci. 2017;25:34-41.
21. Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Effect of simulated pulpal pressure on self-adhesive cements bonding to dentin. Dent Mater. 2008;24:1156-63.

22. Bacchi A, Abuna G, Babbar A, Sinhoreti M, Feitosa V. Influence of 3-month simulated pulpal pressure on the microtensile bond strength of simplified resin luting systems. J Adhes Dent. 2015;17(3):265-71.
23. Mak Y-F, Lai SCN, Cheung GSP, Chan AWK, Tay FR, Pashley DH. Micro-tensile bond testing of resin cements to dentin and an indirect resin composite. Dent Mater. 2002;18(8):609-21.
24. Cardoso M, Moretto S, Carvalho R, Russo E. Influence of intrapulpal pressure simulation on the bond strength of adhesive systems to dentin. Braz Oral Res. 2008;22:170-5.
25. Pashley DH. *In vitro* simulations of *in vivo* bonding conditions. Am J Dent. 1991;4(5):237-40.
26. Hiraishi N, Nishiyama N, Ikemura K, Yau J, King N, Tagami J, *et al*. Water concentration in self-etching primers affects their aggressiveness and bonding efficacy to dentin. J Dent Res. 2005;84:653-8.
27. Uğur M, Altıntaş SH. Evaluation of different desensitizing agents effect on shear bond strength of adhesive resin cement to dentin. J Adhes Sci Technol. 2019;33(15):1695-704.
28. Atay A, Kara O, Kara H, Cal E, Usumez A. Influence of desensitizing procedures on adhesion of resin cements to dentin. J Adhes Sci Technol. 2016;31:1-10.
29. Garcia R, Giannini M, Takagaki T, Sato T, Matsui N, Nikaido T, *et al*. Effect of dentin desensitizers on resin cement bond strengths. RSBO. 2016;12(1):14-22.
30. Thanatvarakorn O, Nakashima S, Sadr A, Prasansuttiporn T, Thitthaweerat S, Tagami J. Effect of a calcium-phosphate based desensitizer on dentin surface characteristics. Dent Mater J. 2013;32(4):615-21.
31. Chow LC. Next generation calcium phosphate-based biomaterials. Dent Mater J. 2009;28(1):1-10.
32. Santana V, de Alexandre R, Rodriques JA, Ely C, Reis A. Effects of immediate dentin sealing and pulpal pressure on resin cement bond strength and nanoleakage. Oper Dent. 2015;41(2):189-99.
33. Vachiramon V, Vargas M, Pashley D, Tay F, Geraldeli S, Qian F, *et al*. Effects of oxalate on dentin bond after 3-month simulated pulpal pressure. J Dent. 2008;36:178-85.
34. Komabayashi T, Spångberg LSW. Particle size and shape analysis of mta finer fractions using portland cement. J Endod. 2008;34(6):709-11.
35. Lenzi TL, Guglielmi Cde A, Arana-Chavez VE, Raggio DP. Tubule density and diameter in coronal dentin from primary and permanent human teeth. Microsc Microanal. 2013;19(6):1445-9.
36. Schilke R, Lisson JA, Bauß O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol. 2000;45(5):355-61.