BJOS
Brazilian Journal of Oral Sciences

Original Article

Volume 22
2023
e237545

Dentin permeability after pretreatment with titanium tetrafluoride and self-etching or universal adhesive systems

Gabriel Greco Franco[1], Ruthinea Faria de Moraes Cardoso[1], Natália Russo Carlos[1], Cecilia Pedroso Turssi[1], Flávia Lucisano Botelho do Amaral[1], Fabiana Mantovani Gomes França[1], Roberta Tarkany Basting[1]

[1] Faculdade São Leopoldo Mandic.

Aim: To evaluate dentin permeability after pretreatment with 2.5% aqueous solution of titanium tetrafluoride (TiF_4), followed by a self-etching universal adhesive system. **Methods:** Forty dentin discs (1.5 mm thick) were randomly divided into groups according to the application or non-application of a pretreatment, and the type of adhesive system to be tested (two-step self-etching/ Clearfil SE Bond/ Kuraray Medical, or universal adhesive system/ Single Bond Universal/ 3M ESPE). Both sides of the discs were conditioned with 37% phosphoric acid to remove the smear layer. The first hydraulic conductivity measurement (L1) was performed in a permeability machine, under 5 PSI pressure. The samples were sanded again to form a standardized smear layer. The teeth designated for pre-treatment with TiF_4 received the TiF_4 solutions applied actively for 60 seconds, and the adhesive systems were applied according to the manufacturer's instructions. Then, a new hydraulic conductivity measurement (L2) was performed for the purpose of calculating the hydraulic conductance at a later time, considering the water viscosity and the thickness of the specimen. The percentage (L) of dentin permeability after application of the adhesive system was obtained (L (%) = [(L1-L2) x100] / L1). The Mann-Whitney non-parametric test was applied. **Results:** There was no difference between the two adhesive systems, or between the groups with or without pretreatment, as regards dentin permeability (p>0.05). **Conclusion:** Pretreatment with 2.5% TiF_4 did not influence dentin permeability, irrespective of the adhesive system used.

Keywords: Dentin permeability. Dentin-bonding agents. Titanium. Fluorides.

Corresponding author:
Profa. Dra. Roberta Tarkany Basting
Faculdade São Leopoldo Mandic
Departamento de Dentística e Materiais Dentários
Rua José Rocha Junqueira, 13.
Bairro Swift, Campinas – SP
CEP: 13045-755 Brazil
Telephone/Fax: 55-19-3211-3600
E-mail: rbasting@yahoo.com

Editor: Dr. Altair A. Del Bel Cury

Received: November 11, 2021
Accepted: April 2, 2022

Introduction

Advancements in the development of products for adhesive restorative systems has allowed direct restorative procedures to be performed with minimally invasive cavity preparations[1]. Although the methods for bonding contemporary adhesive systems to dental substrates are easy to perform, there is always room for improvement in their composition, with the aim of increasing their bond strength to different substrates, and thus increasing the longevity of the restorations[2].

Dentin has dental tubules filled with circulating dentinal fluid, under constant pulp pressure, which keeps the surface of this substrate permanently moist and makes adhesion difficult[3], especially when there are active carious lesions in deep dentin[4]. In self-etching adhesive systems, the tubules remain partially occluded, because of incomplete smear layer removal; hence, the dentin surface is less susceptible to the effects of pulp pressure[5]. Since universal adhesive systems work by way of chemical bonding to the tooth structure, it is questionable whether the tooth permeability resulting from these adhesive systems is lower than it would be with older generation adhesives.

The suggestion has been that some products can be used together with adhesive systems to increase the longevity of the bond to dental tissues, especially dentin, among them chlorhexidine[6], and some phenolic compounds present in green tea[7]. The aforementioned products have been evaluated for their potential of inhibiting endogenous enzymes, preventing proteolytic activity in collagen fibers, and maintaining the hybrid layer for a longer time. Their ability to increase cross-links between collagen fibrils has also been assessed with a view to promoting increased resistance to degradation by endogenous proteases[8].

Titanium tetrafluoride (TiF_4) is an inorganic fluoride compound that has also been researched as a dentin pretreatment to increase the longevity of the hybrid layer[9-11]. TiF_4 is usually used in the form of gel, varnish or aqueous solution, applied to the enamel surface to prevent caries lesions, reduce in vitro demineralization[12], and inhibit the progression of caries lesions in situ and in vivo[13]. It has the ability to prevent erosion and abrasion injuries[14], and to reduce the risk of dentin hypersensitivity[15].

When TiF_4 is hydrolyzed, it acquires a low pH rate, at which titanium tends to bind to an oxygen atom of a phosphate group on the tooth surface[16]. This leads to the formation of a solid element composed of titanium oxides or organometallic complexes, and a stable, modified, acid-resistant smear layer[17,18]. In this case, occlusion of the dentinal tubules occurs[18], which is capable of reducing dentin permeability[19].

When dentin pretreatment with 2.5% TiF_4 was applied, before or after acid etching, followed by the application of a conventional adhesive system, there were no changes in the bond strength to dentin[20], and similar results were reported with self-etching adhesives[9-11]. However, the influence of dentin pretreatment with 2.5% TiF_4 associated with self-etching and universal adhesive systems on dentin permeability have not yet been evaluated. Therefore, the aim of this study was to evaluate dentin permeability after pretreatment with a 2.5% aqueous solution of titanium tetrafluoride (TiF_4), followed by the use of a self-etching of universal adhesive system.

Materials and methods

Sample Preparation

Forty healthy human third molars (approved by the Ethics Committee - CAAE 16187519.6.0000.5374) were used, cleaned with scalpel blades, washed with water, and stored frozen until ready to use. The crowns were sectioned perpendicular to the long axis of the tooth, 1.5 mm above the enamel-cement junction, using a precision saw (Isomet 1000, Buehler, Springfield, VA, USA) under cooling, to obtain the dentin discs[21]. The samples were sanded on both sides with 600 grit sandpaper to ensure uniformity and smoothness of the surfaces[22]. The occlusal surface of the sample was sanded until it was completely free of enamel. A diamond tip was used to mark the occlusal face that was used to adjust the slice of the permeability machine. The thickness of each sample was checked with a digital caliper (Mitutoyo Sul Americana, MIP/E, Suzano, SP, Brazil). The final thickness of the dentin discs was 1.5 mm.

The discs were rinsed with water and stored in a flask with 5 mL distilled water in an incubator for 24 h. After this period, the slices were removed from the flask and first dried with paper for 15 s, and then with air for 5 s. After this, the smear layer was created by removing the polish with 37% phosphoric acid for 15 s[21] and washing with water, on both sides for 30 s. Acid etching was performed[23] to open the dentinal tubules and increase dentin permeability, as a way to ensure standardization of the first hydraulic conductivity measurement (L1) (considered 100%), corresponding to the maximum filtration. Afterwards, the occlusal surfaces were polished with 300 grit sandpaper for 30 s to form a standardized smear layer[22].

A total of 40 samples were prepared and randomly distributed into four groups (n=10). Then they were dried with absorbent paper for 15 s, and received adhesive treatment on the occlusal surface, as specified for the particular group, according to the manufacturer's recommendations.

Dentin treatments

The 40 dentin specimens were separated into groups (n = 10): Pretreatment + Clearfil SE Bond, Pretreatment + Single Bond Universal, Clearfil SE Bond, Single Bond Universal. The composition of the adhesive systems is described in Table 1.

Table1. Adhesive systems evaluated in this study

Materials	Composition	pH	Manufacturer (city, state, country)
Clearfil SE Bond	Primer: HEMA, 10-MDP, hydrophilic aliphatic dimethacrylate, DL- camphorquinone, water, accelerators, dyes Bond: Bis-GMA, HEMA, 10-MDP, hydrophobic aliphatic dimethacrylate, silica, DL-camphorquinone, initiators, accelerators	Bond 1.55 Primer 1.55	Kuraray Medical Inc. (1621 Sakazu, Kurashiki, Okayama, Japan)
Single Bond Universal	HEMA, Bis-GMA, ethanol, water, 10-MDP, silica, copolymer of acrylic and itaconic acid, silane, camphorquinone, dimethylaminobenzoate	2.82	3M ESPE (Sumaré, São Paulo, Brazil)

Bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate

The teeth assigned to receive the dentin pre-treatment were brushed with TiF_4 P.A. (Sigma Aldrich, Saint Louis, MO, USA) dissolved in distilled water to a final concentration of 2.5% (w/v; pH 1.0). The solution was actively applied to the dentin surface using a disposable brush (Microbrush Corporation, Grafton, WI, USA) for 60 s. After this, the adhesive systems for each group were applied according to the respective manufacturer's instructions.

The primer assigned for use with the Clearfil SE Bond adhesive system was actively applied with a microbrush for 20 s, and then air dried for 5 s at a distance of 10 cm. Another microbrush was used to actively apply the adhesive over the primer for 20 s, and air was applied for 5 s immediately afterwards. The Single Bond Universal adhesive system was actively applied for 20 s and received an air spray for 5 s.

The adhesive system was light-cured with a photoactivation device (VALO, Ultradent, UT, USA) for 20 s with a light intensity of 1000 mW/cm^2. Then, a new hydraulic conductivity measurement was performed (L2).

Permeability Test

A permeability machine (THD, Odeme Dental Research, Luzerna, SC, Brazil) was used under 5 PSI pressure, equivalent to 351.54 cmH_2O[23]. The dentin disc was attached to the filtration chamber device, and the machine system was adjusted. The water entered the dentinal tubules and exerted pressure toward the surface. Detachment of the liquid was marked by the difference in location of the air bubble inside the glass microtube of the equipment

Three measurements were performed during continuous movement of the liquid inside the glass microtube, to ensure that the amount of fluid that passed through the sample could be calculate using the following mathematical formula: $Q=(ri^2l)/t$, where Q (µL / min-1) was the amount of liquid passing through the sample, l(cm) was the linear displacement in the glass capillary, t (min) was the time, and ri (cm^2) was the internal measurement of the glass microtube.

The hydraulic conductivity (L) was obtained considering the viscosity of the water and thickness of the constant specimen, L = Q / (AP), where L is the hydraulic conductivity (µL cm^{-2} min^{-1} cmH_2O^{-1}), A (cm^2) was the dentin surface area, and P (cmH_2O) was the pressure imposed. The hydraulic conductivity of each dentin disc was evaluated at two time points: initially, after acid etching (L1), and after applying the adhesive system protocol with or without TiF_4 pretreatment (L2).

The percentage of dentin permeability after application of the adhesive system was obtained using the equation below, with each tooth being its own control: L (%) = [(L1-L2) x100] / L1[24], where L was the percentage of permeability, L1 was the hydraulic conductance after removal of the smear layer, and L2 was the hydraulic conductivity after application of the adhesive system.

Statistical Analysis

Data distribution was evaluated by the Shapiro-Wilk test. Exploratory analysis indicated that the data did not meet the assumptions of analysis of variance (ANOVA). The Mann Whitney non-parametric test was then applied. The results were summarized and presented with median, minimum and maximum values. All the analyses were performed using the R program, with a significance level of 5%.

Results

There was wide variability (%) in permeability among the dentin discs in the same group (Table 2). No significant differences were found between the two groups studied, or between the groups with or without dentin pretreatment, as regards permeability ($p>0.05$).

Table 2. Median (%) (minimum and maximum value) dentin permeability according to application of dentin pretreatment and type of adhesive system

Adhesive System	Dentin Pretreatment		p-value
	None	2.5% TiF_4 aqueous solution	
Single Bond Universal	43.84 (17.68; 81.59)	47.76 (-64.42; 77.32)	0.5967
Clearfil SE Bond	40.26 (-7.57; 71.01)	53.67 (-73.23; 70.84)	0.6501
p-value	0.3258	0.8206	

Discussion

The formation of a hybrid layer with greater permeability causes greater degradation that compromises the tooth-restoration interface and dentinal sealing[25]. Instead, lower permeability of the hybrid layer is desirable because it leads to less elution of resin components[26], and minimum sorption and solubility rates?/levels?/. Nevertheless, dentin pretreatment with 2.5% TiF_4 did not influence dentin permeability, irrespective of the association with either of the two adhesive systems.

Use of TiF_4 in an aqueous solution has been shown to form a layer with a vitreous aspect and promote a reduction in dentin permeability when used in concentrations of 0.1, 0.5, 1.0[27] and 4%[28]. This mechanism has been explained by the occlusion of the dentinal tubules[18], which reduced dentin permeability[19], especially when the smear layer was removed before the application of TiF_4[28]. In contrast, this procedure differed from that of the present study, in which the smear layer was preserved. However, it should be noted that the adhesives used in this study had a mild pH (about 1.55 for Clearfil SE Bond), and ultra-mild (about 2.82 for Single Bond Universal)[29]. It could be suggested that these pH values caused the adhesives to dilute the vitreous layer, especially considering that the adhesive systems were actively applied (by brushing the dentin)[9]. Although Sen and Büyükyilmaz[17](1998) showed that the smear layer treated with TiF_4 was resistant to treatments with 17% EDTA and 5.25% sodium hypochlorite, their results cannot be compared with those of the present study, since the concentration of TiF_4 was 4%, whereas a 2.5% solution was used in the present study.

The ability of self-etching adhesives to establish a mechanically resistant bond to dentin surfaces treated with an aqueous solution of 2.5% TiF_4 has been reported in previous studies[9-11,30], despite this pretreatment solution tending to flocculate, and having a higher-than-average particle size[30]. These adhesive systems have been observed to be capable of penetrating the vitreous layer formed by the application of an aqueous solution of TiF_4. The acidity of the self-etching primer probably caused demineralization of the modified dentin surface layer, leading to the formation of a hybrid layer[9-11,30]. Furthermore, the hydrophilic functional monomer 10-MDP most likely contributed significantly to the chemical bonding to dentin[31].

Single Bond Universal is a single-bottle adhesive, containing hydrophilic components believed to promote greater permeability, by contributing to the formation of a semi-permeable hybrid layer[25]. Whereas Clearfil SE Bond adhesive has fewer hydrophilic components when compared with Single Bond. However, both adhesives are self-etching, a type of system that does not require etching with 37% phosphoric acid. Cruz et al.[32] (2021) also compared etch-and-rinse vs. self-etch adhesive system application modalities and observed similar results to those found in the present study, with no reduction in dentin permeability for Single Bond Universal or Clearfil SE adhesives, irrespective of the application modality. In the present study, both adhesive systems were used without removing the smear layer, hence favoring a decrease in permeability[5,33]. Both adhesives also promoted a chemical interaction with hydroxyapatite through the 10-methacryloxidecyl phosphate monomer (10-MDP), thereby favoring a more stable adhesive interface[34,35]. Thus, the self-etching application mode and the presence of 10-MDP in both adhesive systems may have contributed to their similar permeability level.

The results of the present study suggested that dentin pretreatment with 2.5% TiF_4 did not reduce dentin permeability. However, future studies must be conducted to assess whether this permeability is affected over time, especially in relation to reducing hybrid layer degradation.

In conclusion, the use of 2.5% TiF_4 as a dentin pretreatment, combined with self-etching and universal adhesive systems, did not influence dentin permeability.

Acknowledgements

The authors would like to thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for providing grants (registration number 800280/2018-0).

Data availability

Datasets related to this article are available from the corresponding author upon request.

Conflict of Interest

The authors declare no conflict of interest

Author Contribution

GG Franco: methodology, data acquisition and interpretation, wrote the draft; **RFM Cardoso:** methodology, data acquisition, and data interpretation, wrote the draft;

NR Carlos: data interpretation, revised the draft; **CP Turssi:** data interpretation, revised the draft; **FLB Amaral:** data interpretation, revised the draft; **FMG França:** data interpretation, revised the draft; **RT Basting:** responsible for conception and design, interpretation of the data, wrote and revised the draft. All authors actively participated in the manuscript's findings have revised and approved the final version of the manuscript.

References

1. Alex G. Universal adhesives: the next evolution in adhesive dentistry? Compend Contin Educ Dent. 2015 Jan;36(1):15-26; quiz 28, 40.
2. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011 Jan;27(1):17-28. doi: 10.1016/j.dental.2010.10.023.
3. 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 Jun;23(6):705-13. doi: 10.1016/j.dental.2006.06.010.
4. Mjör IA. Dentin permeability: the basis for understanding pulp reactions and adhesive technology. Braz Dent J. 2009;20(1):3-16. doi: 10.1590/s0103-64402009000100001.
5. Van Meerbeek B, Perdigão J, Lambrechts P, Vanherle G. The clinical performance of adhesives. J Dent. 1998 Jan;26(1):1-20. doi: 10.1016/s0300-5712(96)00070-x.
6. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005 Aug;84(8):741-6. doi: 10.1177/154405910508400811.
7. Carvalho C, Fernandes FP, Freitas Vda P, França FM, Basting RT, Turssi CP, et al. Effect of green tea extract on bonding durability of an etch-and-rinse adhesive system to caries-affected dentin. J Appl Oral Sci. 2016 May-Jun;24(3):211-7. doi: 10.1590/1678-775720150518.
8. Sabatini C, Pashley DH. Mechanisms regulating the degradation of dentin matrices by endogenous dentin proteases and their role in dental adhesion. A review. Am J Dent. 2014 Aug;27(4):203-14.
9. Bridi EC, Amaral FL, França FM, Turssi CP, Basting RT. Influence of dentin pretreatment with titanium tetrafluoride and self-etching adhesive systems on microtensile bond strength. Am J Dent. 2013 Jun;26(3):121-6.
10. Domingues LG, Real CM, Bridi EC, Amaral FLB, França FMG, Turssi CP, et al. Effects of 2.5% TiF_4 on microtensile bond strength: influence of application method and degree of dentin mineralization. Int J Adhes Adhes. 2014 Oct;54:159-64. doi: 10.1016/j.ijadhadh.2014.06.005.
11. Basting RT, Basting RT, Velarde Barrientos S, Bridi EC, França FMG, Turssi CP, et al. Titanium tetrafluoride incorporated into a two-step self-etching adhesive system: physico-mechanical characterization and bonding stability. J Mech Behav Biomed Mater. 2017 Nov;75:197-205. doi: 10.1016/j.jmbbm.2017.07.021.
12. Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF4) varnish on demineralisation and remineralisation of bovine enamel in vitro. J Dent. 2008 Feb;36(2):158-62. doi: 10.1016/j.jdent.2007.12.001.
13. Büyükyilmaz T, Ogaard B, Duschner H, Ruben J, Arends J. The caries-preventive effect of titanium tetrafluoride on root surfaces in situ as evaluated by microradiography and confocal laser scanning microscopy. Adv Dent Res. 1997 Nov;11(4):448-52. doi: 10.1177/08959374970110041101.
14. Wiegand A, Hiestand B, Sener B, Magalhães AC, Roos M, Attin T. Effect of TiF4, ZrF4, HfF4 and AmF on erosion and erosion/abrasion of enamel and dentin in situ. Arch Oral Biol. 2010 Mar;55(3):223-8. doi: 10.1016/j.archoralbio.2009.11.007.

15. Charvat J, Söremark R, Li J, Vacek J. Titaniumtetrafluoride for treatment of hypersensitive dentine. Swed Dent J. 1995;19(1-2):41-6.

16. Tveit AB, Hals E, Isrenn R, Tøtdal B. Highly acid SnF2 and TiF4 solutions. Effect on and chemical reaction with root dentin in vitro. Caries Res. 1983;17(5):412-8. doi: 10.1159/000260695.

17. Sen BH, Büyükyilmaz T. The effect of 4% titanium tetrafluoride solution on root canal walls--a preliminary investigation. J Endod. 1998 Apr;24(4):239-43. doi: 10.1016/S0099-2399(98)80104-0.

18. Basting RT, Leme AA, Bridi EC, Amaral FL, França FM, Turssi CP, et al. Nanomechanical properties, SEM, and EDS microanalysis of dentin treated with 2.5% titanium tetrafluoride, before and after an erosive challenge. J Biomed Mater Res B Appl Biomater. 2015 May;103(4):783-9. doi: 10.1002/jbm.b.33254.

19. Mantilla TF, Silva CVD, Ramos-Oliveira TM, Turssi CP, Ribeiro MS, Freitas PM. TiF4 gel effects on tubular occlusion of eroded/abraded human dentin. Microsc Res Tech. 2017 Nov;80(11):1182-8. doi: 10.1002/jemt.22915.

20. Dündar M, Ozcan M, Cömlekoglu ME, Sen BH. Nanoleakage inhibition within hybrid layer using new protective chemicals and their effect on adhesion. J Dent Res. 2011 Jan;90(1):93-8. doi: 10.1177/0022034510382547.

21. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, et al. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res. 2004 Nov;83(11):843-8. doi: 10.1177/154405910408301104.

22. Pashley DH, Livingston MJ, Greenhill JD. Regional resistances to fluid flow in human dentine in vitro. Arch Oral Biol. 1978;23(9):807-10. doi: 10.1016/0003-9969(78)90159-0.

23. Grégoire G, Guignes P, Nasr K. Effects of dentine moisture on the permeability of total-etch and one-step self-etch adhesives. J Dent. 2009 Sep;37(9):691-9. doi: 10.1016/j.jdent.2009.05.010.

24. Carvalho AO, Oliveira MT, Nikaido T, Tagami J, Giannini M. Effect of adhesive system and application strategy on reduction of dentin permeability. Braz Oral Res. 2012 Sep-Oct;26(5):397-403. doi: 10.1590/s1806-83242012005000020.

25. Giannini M, Makishi P, Ayres AP, Vermelho PM, Fronza BM, Nikaido T, et al. Self-etch adhesive systems: a literature review. Braz Dent J. 2015 Jan-Feb;26(1):3-10. doi: 10.1590/0103-6440201302442.

26. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005 Feb;84(2):118-32. doi: 10.1177/154405910508400204.

27. Kazemi RB, Sen BH, Spångberg LS. Permeability changes of dentine treated with titanium tetrafluoride. J Dent. 1999 Sep;27(7):531-8. doi: 10.1016/s0300-5712(99)00029-9.

28. Calabria M, Porfirio R, Fernandes S, Wang L, Buzalaf M, Pereira J, et al. Comparative in vitro effect of TiF4 to NaF and potassium oxalate on reduction of dentin hydraulic conductance. Oper Dent. 2014 Jul-Aug;39(4):427-32. doi: 10.2341/13-156-L.

29. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater. 2005 Sep;21(9):864-81. doi: 10.1016/j.dental.2005.02.003.

30. Torres GB, da Silva TM, Basting RT, Bridi EC, França FMG, Turssi CP, et al. Resin-dentin bond stability and physical characterization of a two-step self-etching adhesive system associated with TiF4. Dent Mater. 2017 Oct;33(10):1157-70. doi: 10.1016/j.dental.2017.07.016.

31. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007 Sep;28(26):3757-85. doi: 10.1016/j.biomaterials.2007.04.044.

32. Cruz J, Silva A, Eira R, Sousa B, Lopes M, Cavalheiro A. Dentin Permeability and Nanoleakage of Universal Adhesives in Etch-and-rinse vs Self-etch Modes. Oper Dent. 2021 May;46(3):293-305. doi: 10.2341/19-276-L.

33. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, et al. In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM. Biomaterials. 2004 Nov;25(25):5565-74. doi: 10.1016/j.biomaterials.2004.01.009.

34. Yoshida Y, Yoshihara K, Hayakawa S, Nagaoka N, Okihara T, Matsumoto T, et al. HEMA inhibits interfacial nano-layering of the functional monomer MDP. J Dent Res. 2012 Nov;91(11):1060-5. doi: 10.1177/0022034512460396.

35. Fehrenbach J, Isolan CP, Münchow EA. Is the presence of 10-MDP associated to higher bonding performance for self-etching adhesive systems? A meta-analysis of in vitro studies. Dent Mater. 2021 Oct;37(10):1463-85. doi: 10.1016/j.dental.2021.08.014.