Original Article

Effects of Different Storage Media on Dentin Moisture, Microhardness, and Bond Strength of Resin Composite

F Yilmaz, P Guvenc[1], T Pamir[2], A Demirbas, BH Sen[3]

Department of Restorative Dentistry, Faculty of Dentistry, Mugla Sitki Kocman University, Mugla, Turkey, [1]Department of Restorative Dentistry, Faculty of Dentistry, Dokuz Eylul University, Izmir, Turkey, [2]Department of Restorative Dentistry, Faculty of Dentistry, Ege University, Izmir, Turkey, [3]Department of Endodontics, Izmir, Turkey

Abstract

Background: In *in vitro* studies, it is desirable that the storage solutions in which dental samples kept between extraction and experiment should prevent dehydration and have antimicrobial properties. However, it should be taken into consideration that these solutions may have some effects that directly change test results on physical and mechanical properties of laboratory samples. **Aims:** The aim of this *in vitro* study was to evaluate the effects of different storage media on dentin moisture, microhardness, and microshear bond strength of resin composite to dentin. Thirty non-carious human premolars were randomly divided into three groups: 1. 0.1% Thymol (T), 2. Distilled water (DW), 3. Dry storage (DS) (control) (n = 10). Dentin moisture was measured with a digital grain moisture meter. Dentin microhardness was measured with the Vickers test. The bond strength was measured with a microshear test. **Materials and Methods:** Analysis of variance (ANOVA) followed by the Bonferroni test was used for statistical evaluation ($p = 0.05$). **Results:** Dentin moisture of the experimental groups was statistically higher than that of the control group ($p < 0.05$). In addition, the dentin moisture of group DW was significantly higher than that of group T ($p < 0.05$). The mean microshear bond strength of resin composite to dentin was higher in group DW than in group T and group DS ($p < 0.05$), while there was no statistical difference between group T and group DS. The microhardness values of all groups were statistically similar. **Conclusions:** Storage solutions used for disinfection and to prevent dehydration may have negative effects on dentin moisture and bond strength.

Keywords: *Dentin microhardness, dentin moisture, microshear bond strength, storage media*

Received: 27-Jul-2022; **Revision:** 01-Dec-2022; **Accepted:** 02-Jan-2023; **Published:** 27-Feb-2023

Introduction

Currently, the physical properties of adhesive restorative materials, such as dentin bonding strength, surface hardness, color harmony, and microleakage can be tested by *in vitro* studies. Extracted human teeth are often used for these studies.[1,2] However, numerous pathogens have been detected when these teeth are used in the laboratory environment.[3] Therefore, storage solutions should not only keep the specimens moist but also avoid microbial contamination during the period from extraction to the experimental stage.[3]

Distilled water (DW) and normal saline have been used as storage media, although they have no antimicrobial effect.[4] On the other hand, microbial growth is prevented by adding chemical agents such as chloramine, formalin, sodium hypochlorite, thymol, alcohol, and glutaraldehyde to storage solutions because of their bacteriostatic and bactericidal effects.[5] However, it has been previously indicated that chemicals in the solutions change the structure of dental hard tissues and the properties of the materials used in the experiment.[6]

Access this article online	
Quick Response Code:	Website: www.njcponline.com
	DOI: 10.4103/njcp.njcp_498_22

Address for correspondence: Dr. F Yilmaz, Orhaniye Neighbourhood, Haluk Ozsoy Avenue, No: 4, 48000, Mentese, Mugla, Department of Restorative Dentistry, Faculty of Dentistry, Mugla Sitki Kocman University, Mugla/Turkey. E-mail: dt.fatmayilmaz@gmail.com

How to cite this article: Yilmaz F, Guvenc P, Pamir T, Demirbas A, Sen BH. Effects of different storage media on dentin moisture, microhardness, and bond strength of resin composite. Niger J Clin Pract 2023;26:211-6.

It has been observed that optimal dentin moisture increases the bond strength of adhesive systems.[7,8] The bonding of adhesive systems to dentin is more difficult than to enamel because of the presence of dentinal fluid, the dynamics of which depend on intrapulpal pressure.[9] Hydrophilic structures have been added to the chemical ingredients of bonding agents to increase the bond strength of current adhesive systems to dentin and to manage dentinal fluid.[10] Furthermore, during the adhesive application, the removal of dentinal fluid around the collagen fibers in the organic structure of dentin negatively affects the bond to dentin due to excessive drying and subsequent shrinkage of the collagen fibers.[11] Therefore, maintaining dentin moisture in equilibrium is a difficult situation, which requires technical precision.[11,12] To test the bond strength of adhesive systems, it is more rational to evaluate the bonding to dentin rather than to enamel. Although the dentinal fluid is the primary source of dentin moisture in vital teeth during clinical use of adhesive systems, extracted teeth used for *in vitro* studies do not have this fluid and lack oral moisture and soft tissue. In *in vitro* studies, the moisture ratio in dentin has been found to affect both the resin-dentin interface and the structural features of dentin.[13-15] However, there are not enough studies in the literature on how dentin moisture is affected by the storage media in dentin bond strength studies.

The traditional method that involves desiccation of samples to calculate weight change has been used to measure dentin moisture but it causes irreversible loss of samples and is time-consuming.[16] On the other hand, a new technique that provides more accurate information for measuring dentin moisture was developed in 1990,[17] initially to estimate the moisture content of a single peanut kernel. In this method, the moisture value is measured by placing the kernel between two plates connected to a parallel plate capacitor.[17] The digital grain moisture meter is a commercial device that uses impedance technology, and it has been calibrated using samples with known moisture ratios to measure the impedance of various grains.[18] The meter has shown high validity for five-grain modes (paddy, processed long rice, processed short rice, long brown rice, and wheat).[19] Although grains and teeth are structurally different, a pilot study has shown that moisture measurement by impedance technology can be successfully used in dentin samples.[19] For this reason, using a grain moisture meter could reliably measure the moisture ratio of dental tissues without destructing the samples.

The aim of this *in vitro* study was to evaluate the effects of 0.1% thymol, DW, and dry storage (DS) as storage media on dentin moisture, microhardness, and microshear bond strength of resin composite to dentin.

The null hypotheses of this study are as follows: 1. There is no difference among the dentin moisture ratio of samples stored in different media; 2. There is no difference among the dentin microhardness values of samples stored in different media; and 3. There is no difference among the microshear bond strength of samples stored in different media.

Subjects and Methods

Study design

After 30 non-carious human premolars extracted for orthodontic reasons were randomly numbered, they were matched as three. Matching teeth were ordered from smallest to largest according to their numbers. The assignment of the subjects to the groups (0.1% thymol (T) or DW as experimental groups and DS as a control group (n = 10)) was made according to the randomization table (www.random.org). The thymol solution and DW were prepared in the laboratory. After 7 days in the assigned storage solution at room temperature, teeth were separated mesiodistally into two halves using a diamond disc (Meisinger GmbH, Neuss, Germany) under water-cooling. After the enamel was removed with diamond burs (Meisinger GmbH, Neuss, Germany) to expose the dentin to 2.2 mm thick, the roots were cut at the cementoenamel junction. The lingual halves of the samples were used for dentin moisture measurements and the buccal halves of the samples were used for dentin microhardness measurements and for microshear bond strength of a resin composite to dentin.

In the first stage of this study, a novel non-destructive method was used to measure dentin moisture. Dentin moisture of the samples of 4 mm width, 4 mm length, and 2.2 mm thickness was determined with a digital grain moisture meter (Grain Moisture Meter Riceter M 409, Kett, CA, US) in wheat mode by measuring 9 times according to the manufacturer's instructions. The data were recorded as percent moisture.

In the second stage, the samples were prepared for surface hardness measurements. The dimension of dentin samples was standardized in a square format with a width of 4 mm, a length of 4 mm, and a thickness of 2.2 mm. Then, the samples were numbered and placed in transparent acrylic blocks (Imicryl Dental, Konya, Turkey). The buccal sides of the exposed dentin surfaces were sanded with 180, 240, 400, 600, 1000, 1500, and 2000 grit SiC sandpaper under copious water-cooling in a polishing device (Panambra DP-10; Panambra Industriale Tecnica S.A., Sao Paulo, SP, Brazil) to obtain a flat dentin surface with a standardized smear layer, respectively. The surfaces were polished with green and white dental felt. Then, the samples were ultrasonically cleaned in deionized water for 10 min and kept in an incubator at 37°C until microhardness test measurements. Each sample was placed on the Vickers microhardness tester (HMV-2 Microhardness Tester, Shimadzu, Japan) and a load of 1 N was applied for 15 s.[20] At least three measurements were made in each sample with approximately 200 µm distance between the indentations, and the mean microhardness value was calculated using the formula: $HV = 1.854 \times F / d^2$. The applied load is "F (kgf)," and right after the indentation, a diagonal length of the pyramid shaped impression on the substrate is "d (mm)". Vickers hardness value was given in N/mm^2.[20]

After the microhardness test, the microshear bond strength test was performed. A two-step self-etch adhesive resin (Clearfil SE Bond, Kuraray Noritake Dental Inc., Okayama, Japan) was applied to the dentin surfaces according to the manufacturer's instructions and polymerized for 10 s with a light curing unit (EliparTM S10, 3M ESPE, USA). Tygon tubes 3 mm long and 0.9 mm in diameter were placed in the center of the dentin surface and then filled with resin composite (Clearfil Majesty Posterior, Kuraray Noritake Dental Inc., Okayama, Japan). Resin composite was applied in 2-mm incremental layers, and each layer was polymerized for 20 s. The Tygon tubes filled with resin composite were then removed with pliers from the surface of the dentin samples in which they were placed. However, layering, polymerization, or adhesion to the dentin of the resin composite in the tube required high technical precision. In some samples, resin composite fractures or ruptures were observed. Because it has only a 0.9 mm diameter, polymerized resin composite was easily broken during the removal of the tubes. Similarly, all damaged samples were excluded from the study and the samples were renewed. Then, the samples were placed in a microshear testing device. A 0.2 mm diameter wire was looped halfway around the resin cylinder. The wire loop, resin/dentin interface, and load cell were approximately brought straight into line. The shear force was applied at a rate of 1 mm/min speed with a mini universal testing machine (Harvard Apparatus Co. Inc., Dover, MA, USA). The mean values of microshear bond strength were given in MPa.

Statistics

For statistical evaluation, the SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) was used. Data were first evaluated using the Kolmogorov–Smirnov test and found to have a normal distribution. Thereafter, analysis of variance (ANOVA) and Bonferroni tests were used to test the statistical differences among groups ($\alpha = 0.05$).

Table 1: The effect of different storage media on dentin moisture

Storage Media	Thymol	Distilled Water	Dry Storage (Control)
MP (Mean±SD)	14.04%[b]±0.49	18.46%[a]±2.07	11.62%[c]±0.7

Different letters in the superscript along the rows indicate statistically significant differences (P<0.05); MP: Moisture Percentages, SD: Standard Deviation

Table 2: The effect of different storage media on dentin microhardness

Storage Media	Thymol	Distilled Water	Dry Storage (Control)
HV (Mean±SD)	69.43[a]±5.52	70.74[a]±3.86	70.04[a]±5.17

The same letter in the superscript along the rows indicates statistically similarity (P>0.05); HV: Vickers Hardness, SD: Standard deviation

Table 3: The effect of different storage media on the microshear bond strength of composite to dentin

Storage Media	Thymol	Distilled Water	Dry Storage (Control)
MPa (Mean±SD)	19.94[b]±4.20	50.87[a]±0.35	29.31[b]±2.19

Different letters in the superscript along the rows indicate statistically significant differences (P<0.05); MPa: Megapascal, SD: Standard Deviation

Results

The dentin moisture was affected by different storage media ($p < 0.05$). The moisture percentages of the samples in both experimental groups (T and DW) were significantly higher than those of the control group (DS) ($p < 0.05$). In addition, the values of group DW were higher than those of group T ($p < 0.05$) [Table 1].

The microhardness of the dentin surface was not affected by different storage media. There was no statistically significant difference among microhardness values of all groups ($p > 0.05$) [Table 2]. On the other hand, the bond strength of resin composite to dentin was affected by the different storage media ($p < 0.05$). Group DW showed statistically higher bond strength values than group T and group DS ($p < 0.05$). There was no statistically significant difference between group T and group DS [Table 3].

Discussion

Traditional techniques for measuring dentin moisture require drying the specimens to calculate the change in weight. This method is time-consuming and results in the irreversible destruction of the samples.[16] On the other hand, a grain moisture meter as a new technique has been suggested to measure dentin moisture more accurately. The present data show that the grain moisture meter can also be used for dentin moisture measurements under *in vitro* conditions as well as different grains. In this study, a grain moisture meter with the same impedance technology was used in wheat mode, which allows non-destructive measurement of dentin moisture.

There was a significant difference in the moisture percentage of the dentin samples stored in thymol, DW, or dry environments. In groups T and DW, dentin moisture values were significantly higher than that of the control group ($p < 0.05$), and the first null hypothesis was rejected. The values of group DW were also higher than those of group T ($p < 0.05$). Since thymol, an aromatic oil derived from plants, has demineralization and oxidizing effect, it may have changed the dentin moisture when used as a storage solution. The collagen fibers tend to collapse in demineralized dentin and thus reducing permeability. As a result of the decrease in permeability on the demineralized area, moisture loss may have occurred in dentin in group T. On the other hand, since the DW storage solution did not cause any demineralization of dentin, moisture loss may also have been relatively unobserved when compared to the group T. Similarly, an *in vitro* study[16] reported that the moisture in root dentin of extracted human teeth kept in different storage solutions was increased in all tested media. On the other hand, in another study,[21] it was found that water loss from dentin was not affected by different storage media for 3 days while it was significantly affected after 12-day storage. It was also recommended that minimum storage time in neutral-buffered formalin minimally changed dentin after extraction.[21] In this study, the samples were kept in the storage solutions for 7 days. In another study[22] evaluating changes in root dentin moisture, the samples stored in unreplenished ascorbic acid solutions or DW for longer than 3 days did not result in a further increase in dentin moisture. All these results show that storage time affects dentin moisture.

Microhardness is the gold standard parameter used to observe changes in mineralized tissues and predict other important mechanical properties.[23] Surface hardness is expressed as resistance to continuous pressure from a conical or spherical tip based on the indentation left on the surface.[24] Dentin has a Knoop hardness of 68 kg/mm^2[2,25] and it is directly proportional to the amount of calcified matrix per mm.[2,26] Microhardness is affected by the composition of hard tissues, surface structure, and mineral uptake or loss.[27,28] The overall decrease in dentin hardness is caused by the decrease in the hardness of the intertubular matrix as a result of the heterogeneous distribution of the mineral phase in the collagen matrix.[27] The microhardness of dentin is affected by some factors such as demineralization by acidic chemicals, organic dissolution of collagen-rich intertubular dentin, heat-induced changes, and ion exchanges.[26-29] In this study, a load of 1 N was applied for 15 s because it has been determined that cracks may occur on the surface of the samples if an excessive load is applied to elastic materials and this may lead to incorrect results.[24] Some studies[20,30] indicate that storage conditions change the mechanical properties of dental tissues. It was reported that teeth stored for 12 months had a decrease in the microhardness of both enamel and dentin.[20] In addition, it was found that the decrease in microhardness was relatively greater in teeth stored in glutaraldehyde than in teeth stored in deionized water and thymol solutions. In contrast, there was no statistical difference between dentin microhardness values in the experimental and control groups in this study. It can be explained by some several possible mechanisms. Firstly, the dentin surfaces were not exposed when the extracted tooth samples were kept in storage media. The microhardness test was performed after storage and enamel removal from the tooth samples. The enamel layer may have acted as a barrier preventing the infiltration of solutions into the underlying dentin and/or the mineral loss from the dentin. The other reason may be relevant to storage time. The 7-day storage period may not have been effective in reducing the microhardness. In addition, as a result of the limited dissolution of ions such as calcium and phosphate from cross-linked organic material, a similar result may have occurred in the experimental and control groups. As a result, the second null hypothesis was accepted. The different results in microhardness changes between this study and one study[20] could be due to the different storage times. It should also be noted that microhardness measurement results are affected by the thickness of the dental tissue, so that different results may occur for samples with different thicknesses.[27,31] The thickness of the sample can be related to the tooth structure. In *in vitro* study, different samples have been used such as premolar or molar teeth. In addition, the mineral content of these samples, as well as their thickness, may differ from each other. Furthermore, there may be an increase in dentin microhardness after storage, which can be explained by the limited transport of calcium and phosphate ions.[32] Similarly, it was reported that the microhardness of teeth immersed in 2% glutaraldehyde for 20 min decreased by 15%, whereas it increased by 15% after 2 days.[33]

In studies investigating the effects of storage solutions on bond strength, different results were obtained depending on the type of solution and the duration of storage.[34-38]

According to the data from this study, the third null hypothesis was rejected because there were significant differences between the microshear bond strength of samples stored for 7 days in DW and thymol or DW and dry environment. The mean microshear bond strength of group DW was significantly higher than those of group T and group DS. However, some previous studies reported that storage media and time did not affect bond strength values.[34,35] It was indicated that 7- and 30-day storage time did not affect microtensile bond strength, irrespective of the solution type, while formalin and thymol had a negative effect on the bond strength when teeth were stored for 6 months.[36] On the other hand, one study[37] reported that the storage condition influences the long-term durability of dentin bonding with resin cement. In another study, the bond strength of resin composite to enamel stored in thymol was found significantly lower than that of the samples stored in DW.[38] These studies support the results of the current study. The negative effect of storage media on bond strength may depend on several factors. One of them could be related to dentin moisture, as teeth may lose some moisture after being extracted, which cannot be recovered even if teeth are stored in solutions. In this regard, the collagen fiber network in dentin plays the main role. By volume, dentin consists of about 50% apatite crystals, 30% collagen, and 20% water. Collagen fibers collapse after the loss of water around them. As a result, resin infiltration by the bonding agent is compromised and the hybrid layer is poorly formed. The poor hybrid layer results in lower bond strength of composite and adhesive resins to dentin. In this study, teeth stored in thymol solution or dry environment showed both lower dentin moisture and bond strength values than those stored in DW. Similarly, a recent study[39] reported that dentin surface moisture is still an important factor for optimal bond strength. Another reason is the solution type and its effects on resin composites. It was reported that thymol inhibits methacrylate polymerization by reacting with its free radical groups.[40] In this study, the lower bond strength values of teeth stored in thymol compared to those in DW may be attributed to the negative effects of thymol on methacrylate polymerization.

Both microhardness measurements and bond strength tests were performed on the same samples. The force applied during microhardness measurements is likely to produce changes in the dentin surface that will affect the subsequent bond strength. However, the comparative results are considered to be instructive since the procedures are applied in the same way in all samples. In addition, the difficulties encountered in the preparation of samples in a standard way during *in vitro* studies are a limitation of this study, also. Due to morphological differences in maxillary and mandibular premolars and variations in enamel and dentin thickness, it is likely that the remaining dentin thickness in teeth with less enamel thickness will also change. This makes standard dentin samples difficult to access and may increase the standard deviation between data. To obtain correct results in laboratory experiments, teeth samples should be selected from the same area of the dental arch. Furthermore, the samples should be properly prepared and stored immediately after extraction. It is desirable that storage media do not cause any change in the physical and mechanical properties such as a decrease in microhardness and moisture of the dental tissues and ensure that harmful microorganisms on the extracted teeth are killed to prevent cross-infection. However, it has been reported that storage solutions used as disinfectant media damage tooth tissues. Similarly, in this study, a 0.1% thymol solution was found to both reduce dentin moisture and weaken the bond strength of resin composite to dentin compared to DW. Unfortunately, DW has no disinfecting or sterilizing effects on tooth samples although it has no negative effect on dentin moisture, microhardness, and bond strength.

Conclusion

It should be remembered that storage solutions used for disinfection and to prevent dehydration may have negative effects on dentin moisture and bond strength. To ensure the safety and reliability of laboratory studies, storage solutions that will prevent physical and chemical damage such as moisture, hardness, and surface properties to extracted tooth samples, and disinfect at the same time are needed. Planning further studies on the subject will provide a suitable solution.

Acknowledgements

The authors acknowledge the Scientific Research Projects Unit of Ege University (Grant number: 2010-DIS-022) as a funding source for this research study.

Financial support and sponsorship

Scientific Research Projects Unit of Ege University (Grant number: 2010-DIS-022).

Conflicts of interest

There are no conflicts of interest.

References

1. Pashley DH. *In vitro* simulations of *in vivo* bonding conditions. Am J Dent 1991;4:237-40.
2. Goodis HE, Marshall GW Jr, White JM, Gee L, Hornberger B, Marshall SJ. Storage effects on dentin permeability and shear bond strengths. Dent Mater 1993;9:79-84.
3. Schulein T. Infection control for extracted teeth in the teaching

laboratory. J Dent Educ 1994;58:411-3.

4. Camps J, Baudry X, Bordes V, Dejou J, Pignoly C, Ladeque P. Influence of tooth cryopreservation and storage time on microleakage. Dent Mater 1996;12:121-6.
5. NawrockaA A, Łukomska-Szymańska M. Extracted human teeth and their utility in dental research. Recommendations on proper preservation: A literature review. Dent Med Probl 2019;56:185-90.
6. Ziskind D, Gleitman J, Rotstein I, Friedman M. Evaluation of cetylpyridinium chloride for infection control in storage solution. J Oral Rehabil 2003;30:477-81.
7. Tay F, Gwinnett J, Wei SH. Relation between water content in acetone/alcohol-based primer and interfacial ultrastructure. J Dent 1998;26:147-56.
8. de Siqueira FSF, Pinto TF, Carvalho EM, Bauer J, Gonçalves LM, Szesz AL, *et al*. Influence of dentinal moisture on the properties of universal adhesives. Int J Adhes Adhes 2020;101:102633.
9. Kanca J III. Resin bonding to wet substrate. I. Bonding to dentin. Quint Int 1992;23:39-41.
10. Van Meerbeek B, Yoshida Y, Lambrechts P, Vanherle G, Duke ES, Eick JD, *et al*. A TEM study of two water-based adhesive systems bonded to dry and wet dentin. J Dent Res 1998;77:50-9.
11. Kanca J 3rd. Wet bonding: Effect of drying time and distance. Am J Dent 1996;9:273-6.
12. Reis A, Loguercio AD, Carvalho RM, Grande RHM. Durability of resin dentin interfaces: Effects of surface moisture and adhesive solvent component. Dent Mater 2004;20:669-76.
13. Al Qahtani MQ, Platt JA, Moore BK, Cochran MA. The effect on shear bond strength of rewetting dry dentin with two desensitizers. Oper Dent 2003;28:287-96.
14. Gallo J 3rd, Henderson M, Burgess J. Shear bond strength to moist and dry dentin of four dentin bonding systems. Am J Dent 2000;13:267-70.
15. Van Meerbeek B, Dhem A, Goret-Nicaise M, Braem M, Lambrechts P, VanHerle G. Comparative SEM and TEM examination of the ultrastructure of the resin-dentin interdiffusion zone. J Dent Res 1993;72:495-501.
16. Komabayashi T, Ahn C, Zhang S, Zhu Q, Spångberg LSW. Chronologic comparison of root dentin moisture in extracted human teeth stored in formalin, sodium azide, and distilled water. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e50-4.
17. Kandala C, Nelson S. Measurement of moisture content in single kernels of peanuts: A nondestructive electrical method. Trans ASABE 1990;33:567-71.
18. Kandala CV. Moisture determination in single peanut pods by complex RF impedance measurement. IEEE Trans Instrum Meas 2004;53:1493-6.
19. Komabayashi T, Zhu Q, Jiang J, Safavi KE, Spångberg LSW. A rapid nondestructive method for root dentin moisture measurements: *In vitro* pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:e107-11.
20. Aydın B, Pamir T, Baltaci A, Orman MN, Turk T. Effect of storage solutions on microhardness of crown enamel and dentin. Eur J Dent 2015;9:262-6.
21. Jameson M, Tidmarsh B, Hood J. Effect of storage media on subsequent water loss and regain by human and bovine dentine and on mechanical properties of human dentine in vitro. Arch Oral Biol 1994;39:759-67.
22. Komabayashi T, Ahn C, Zhang S, Zhu Q, Spångberg LSW. Root dentin body moisture changes caused by ascorbic acid. J Dent 2009;37:475-9.
23. Currey J, Brear K. Hardness. Young's modulus and yield stress in mammalian mineralized tissues. J Mater Sci Mater Med 1990;1:14-20.
24. Zamfirova G, Dimitrova A. Some methodological contributions to the Vickers microhardness technique. Polym Test 2000;19:533-42.
25. Craig R, Peyton F. The microhardness of enamel and dentin. J Dent Res 1958;37:661-8.
26. Marshall GW Jr, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: Structure and properties related to bonding. J Dent 1997;25:441-58.
27. Tesch W, Eidelman N, Roschger P, Goldenberg F, Klaushofer K, Fratzl P. Graded microstructure and mechanical properties of human crown dentin. Calcif Tissue Int 2001;69:147-57.
28. Strawn SE, White JM, Marshall GW, Gee L, Goodish HE, Marshall SJ. Spectroscopic changes in human dentine exposed to various storage solutions—short term. J Dent 1996;24:417-23.
29. Kinney JH, Balooch M, Marshall SJ, Weihs TP. Hardness and Young's modulus of human peritubular and intertubular dentine. Arch Oral Biol 1996;41:9-13.
30. Muhleman H. Storage medium and enamel hardness. Helv Odont Acta 1964;8:112-7.
31. Alrabii SA, Zumot LY. Chip thickness and microhardness prediction models during turning of medium carbon steel. J Appl Math 2007;2007:051905.
32. Arends J, Ögaard B, Ruben J, Wemes J, Rölla G. Influence of glutardialdehyde on dentin demineralization *in vitro* and in vivo. Eur J Oral Sci 1989;97:297-300.
33. Wemes J, Arends J. The hardness of bovine dentine after glutaraldehyde treatment. Oral Surg Oral Med Oral Pathol 1984;58:722-4.
34. Aquilino S, Williams V. The effect of storage solutions and mounting media on the bond strenghts of a dentinal adhesive to dentin. Dent Mater 1987;3:131-4.
35. Jörgensen KD, Itoh K, Munksgaard EC, Asmussen E. Composite wall-to-wall polymerization contraction in dentin cavities treated with various bonding agents. Eur J Oral Sci 1985;93:276-9.
36. Santana FR, Pereira JC, Pereira CA. Influence of method and period of storage on the microtensile bond strength of indirect composite resin restorations to dentine. Braz Oral Res 2008;22:352-7.
37. Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on dentin bond durability of resin cement. Dent Mater 2000;16:1-6.
38. Tosun G, Sener Y, Sengun A. The effect of various storage solutions on the bond strength of resin composite to enamel. Clin Dent Res 2005;29:2-6.
39. Tsujimoto A, Shimatani Y, Nojiri K, Barkmeier WW, Markham MD, Takamizawa T, *et al*. Influence of surface wetness on bonding effectiveness of universal adhesives in etch-and-rinse mode. Eur J Oral Sci 2019;127:112-9.
40. Fujisawa S, Kadoma Y. Effect of phenolic compounds on the polymerization of methyl methacrylate. Dent Mater 1992;8:324-6.