UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM
ODONTOLOGIA (PPGO) - MESTRADO

VICTOR PATRICK OENNING

Avaliação da resistência de união adesiva à dentina em diferentes tempos e soluções de armazenamento

Cascavel-PR
2019

VICTOR PATRICK OENNING

Avaliação da resistência de união adesiva à dentina em diferentes tempos e soluções de armazenamento

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, como requisito parcial para obtenção do título de Mestre em Odontologia.

Área de concentração: Odontologia.

Orientador: Profª. Drª. Vera Lucia Schmitt.

Cascavel-PR
2019

Ficha de identificação da obra elaborada através do Formulário de Geração Automática do Sistema de Bibliotecas da Unioeste.

Oenning, Victor Patrick Avaliação da resistência de união adesiva à dentina em diferentes tempos e soluções de armazenamento / Victor Patrick Oenning; orientador(a), Vera Lucia Schmitt, 2019.
23 f.

Dissertação (mestrado), Universidade Estadual do Oeste do Paraná, Campus de Cascavel, Centro de Ciências Biológicas e da Saúde, Programa de Pós-Graduação em Odontologia, 2019.

1. cisalhamento. 2. dentina. 3. timol. I. Schmitt, Vera Lucia. II. Título.

unioeste
Universidade Estadual do Oeste do Paraná
Campus de Cascavel CNPJ 78680337/0002-65
Rua Universitária, 2069 - Jardim Universitário - Cx. P. 000711 - CEP 85819-110
Fone:(45) 3220-3000 - Fax:(45) 3324-4566 - Cascavel - Paraná

PARANÁ
GOVERNO DO ESTADO

VICTOR PATRICK OENNING

Avaliação da resistência de união adesiva à dentina em diferentes tempos e soluções de armazenamento

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia em cumprimento parcial aos requisitos para obtenção do título de Mestre em Odontologia, área de concentração Odontologia, linha de pesquisa Materiais Dentários Aplicados À Clínica Odontológica, APROVADO(A) pela seguinte banca examinadora:

Orientador(a) - Vera Lucia Schmitt
Universidade Estadual do Oeste do Paraná - Campus de Cascavel (UNIOESTE)

Christian Giampietro Brandão
Universidade Estadual do Oeste do Paraná - Campus de Cascavel (UNIOESTE)

Larissa Pinceli Chaves
Universidade Paranaense - Cascavel (UNIPAR)

Cascavel, 8 de fevereiro de 2019

Avaliação da resistência de união adesiva à dentina em diferentes tempos e soluções de armazenamento

RESUMO

Objetivo: Avaliar o efeito do armazenamento de dentes bovinos em quatro diferentes soluções (água destilada, soro fisiológico, cloramina T 0,5% e timol 0,1%), quando congeladas, por quatro diferentes tempos (uma semana, um, três e seis meses), na resistência de união adesiva à dentina. Metodologia: 136 incisivos bovinos foram divididos em 16 grupos experimentais (n=8), e comparados àquela observada em dentes imediatamente extraídos (grupo controle), totalizando 17 grupos. Os corpos-de-prova foram confeccionados com adesivo convencional Adper Single Bond 2 (3M ESPE, St Paul, MN, EUA) e compósito Opallis Flow (Dentscare LTDA, Joinville, SC, BRA) após os diferentes períodos de armazenamento. Após 7 dias, as unidades amostrais foram testadas em máquina universal de ensaios (EMIC DL 500, São José dos Pinhais, PR, BRA); a força de ruptura foi registrada em N e, de acordo com a área da interface adesiva, convertida em MPa. Resultados: Os grupos armazenados em água destilada apresentaram os menores valores em todos os tempos, e a cloramina T 0,5% mostrou-se a solução mais estável até três meses. Conclusão: Pode-se concluir que diferentes soluções e tempos de armazenamento interferem na resistência da união adesiva à dentina.

Palavras-chave: Cisalhamento, Timol, Dentina.

Evaluation of bond strength to dentin at different times and storage solutions

ABSTRACT

Purpose: To evaluate the effect of bovine teeth storage in 4 different solutions: distilled water (DW), saline (S), 0.5% chloramine (C) and 0.1% thymol(T), when frozen for 4 different times: 1 week, 1 month, 3 and 6 months, on bond strength to dentin. Materials and Methods: 136 bovine incisors were divided into 16 experimental groups (n = 8), and compared to the one observed in immediately extracted teeth that were not stored, the control group (CG), totaling 17 groups. The specimens were made with conventional adhesive Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) and Opallis Flow composite (Dentscare LTDA, Joinville, SC, BRA) after different storage periods. After 1 week, the specimens were tested in a universal testing machine (EMIC DL 500, São José dos Pinhais, PR, BRA); the rupture force was recorded in N and, according to the area of the adhesive interface, converted into MPa. Results: The groups stored in DW had the lowest values at all times, and 0.5% chloramine was the most stable solution up to 3 months. Conclusion: It can be concluded that different solutions and storage times interfere on the bond strength to dentin.

Keywords: Shear Strength, Thymol, Dentin.

Dissertação elaborada e formatada conforme as normas das publicações científicas:
The Journal of Adhesive Dentistry
Disponível em:
https://jad.quintessenz.de/index.php?doc=guidelines

SUMMARY

1. Introduction 8
2. Material and Methods 10
3. Results. 12
4. Discussion 13
5. Conclusion 16
6. Tables 17
7. References 19

Evaluation of bond strength to dentin at different times and storage solutions

1. Introduction

In order to perform laboratory dental studies, researchers constantly use human teeth for *in vitro* tests to check the efficacy of different materials or techniques.[15, 7, 9, 18, 26] However, extracted human teeth have been increasingly difficult to obtain due to the preventive advances of dentistry, such as reduction of missing and decayed teeth.[22, 30]

Thus, bovine teeth may be used as a substitute for human teeth, and they are used extensively in dental studies such as the investigation of proteolytic enzyme activities in dentin,[32,6] bond strength,[4,19] remineralization of carious lesions,[5] color changes,[33] tooth whitening,[28] photopolymerization,[4] among others.

The use of bovine substrate allows standardizing age, sclerosis, and amount of abrasion. Studies have shown no significant differences between human and bovine teeth in terms of bond strength.[3] In addition, scanning electron microscopy observations showed that human and bovine dentin substrates are similar, with approximately the same number, density, and diameter of dentinal tubules, especially in medium-depth dentin.[3]

Thus, as soon as bovine or other teeth are extracted, the means of storage should preserve their properties as closely as possible to the *in vivo* condition.[8, 31] Several storage solutions are reported in the literature for this purpose, such as deionized water, distilled water[9], 0.1% thymol,[24, 32, 6, 26] silver nitrate with ammonia,[15] 0.5% chloramine-T,[7, 18, 4,19] among others. However, storage conditions may change the mineral and/or organic content of teeth[31] and the test performed may present negligible results.

An example of using bovine teeth is to test adhesive systems. The adhesion of resin to dentin occurs through the infiltration and polymerization of hydrophilic resins in the collagen mesh exposed due to the application of acid, forming a hybrid layer. Since their introduction, adhesive systems have been subjected to modifications in their formulations and action mechanisms, aiming to improve bond strength values, especially in dentin.[23]

The *in vitro* methodology most commonly used for determining the efficacy of adhesive systems is microtensile or micro-shear bond strength tests. However, the variability of results has suggested the hypothesis of deficiency in standardizing the technique, complicating the correct interpretation and the comparison with other studies. Among the variables that may influence the results are storage of teeth before or after the inclusion of specimens, time and type of surface acid etching, loading cell velocity in the test machine, among others.[10]

The micro-shear test has been recommended to analyze small areas of adhesive interface, because the step involving the slicing of samples in the shape of toothpicks or hourglasses is not necessary as in microtensile tests. In addition, the method allows obtaining several specimens from a sample of enamel, dentin, or other substrate, because the bonding surfaces are very small (approximately 0.4 mm^2) and they are versatile and useful to assess the bond strength between mineralized tissues and polymeric restorative materials. The preparation of specimens may be standardized, as this is an important factor in the performance of bond durability studies for both conventional and self-etch adhesive systems.[12]

Therefore, this study aimed to assess the effect of bovine teeth storage in four different solutions (DW, S, C, and T) when frozen at four different times (one week and one, three, and six months) on the bond strength to dentin when compared to that observed in immediately extracted teeth (CG).

2. Materials and Methods

Experimental Design

This study had a 2x2 factorial design, with the following factors studied: 1- Storage solution in four levels: distilled water (DW), saline solution (S), 0.5% chloramine-T (C), and 0.1% thymol (T); and 2- Storage time in four levels: one week and one, three, and six months (Table 1). The response variable is the strength of the adhesive bond by means of a micro-shear mechanical test expressed in MPa.

Selection and preparation of teeth

A total of 136 freshly extracted bovine incisors were used. The teeth were sectioned at the crown-root junction with a flexible double-sided diamond disc (ref. 7016. KG Sorensen, Barueri, SP, Brazil) under refrigeration, discarding the roots. The teeth were divided into one control group and 16 experimental groups stored in a freezer (-4 ºC) for one week and one, three, and six months in the following solutions: DW, S, C, and T (Table 1).

After the storage time of the experimental groups, aided by #600 silicon carbide sandpapers (Carborundum, Vinhedo, SP, BRA) assembled in a water-cooled, horizontal, and rotating electrical polishing machine (Model APl-4, Arotec, Cotia, SP, Brazil), the buccal surface of enamel was worn until exposing an area of approximately 16 mm² of dentin.

Specimen preparations

The teeth were fixed with an epoxy resin (Henkel LTDA, Itapevi, SP, Brazil) in PVC tubes filled with chemically activated acrylic resin, maintaining the dentine surface parallel to the PVC device with a parallelometer. After 37% phosphoric acid etching for 15 seconds (Dentscare LTDA, Joinville, SC, Brazil), washing for 30 seconds, and controlling dentin humidity, two layers of adhesive system (Adper Singlebond, 3M ESPE, St. Paul, USA) were applied and photopolymerized for 10 seconds. Then, four transparent cylindrical matrices (Labor Import catheter, Jaipur, India) with their internal volume (0.7 mm) filled with Opallis Flow composite (Dentscare LTDA, Joinville, SC, Brazil) were placed in the healthy dentin of each sample unit (median region of the tooth) on a perforated adhesive tape with four holes of the same diameter as the transparent matrices. The matrices were photopolymerized for 40 seconds (Valo, Ultradent, Indaiatuba, SP, Brazil) with wavelength of 395-480 nm and power of 3200 mW/cm². Then, they were removed with #12 scalpel blades to expose the small composite cylinders (0.7 mm of diameter by 1.0 mm of height) attached to the dentin surface, and stored in 37ºC distilled water for seven days. Table 2 describes the details of materials used in the preparation of specimens.

Micro-shear test (μSBS)

After seven days of resin maturation, the micro-shear test was performed in a universal testing machine (EMIC DL 500, São José dos Pinhais, PR, Brazil). The shear load was applied at the base of the cylinders with a steel wire (0.1 mm of diameter) at speed of 0.5 mm/min until bond rupture. The mean of the four readings, in N (rupture force), was taken for each sample unit.

Control group

In the control group, the teeth were not stored and specimens were prepared immediately after the extraction of teeth, so that storage would not interfere with the results of this group. The micro-shear mechanical test was performed after seven days of specimen preparations, as the experimental groups.

Statistical analysis

The results of the experimental groups were converted into MPa and compared statistically to each other using ANOVA and Tukey's test at 5% significance level, and each experimental group was compared with the control group using the t-test (5%).

3. Results

Regarding the different solutions, distilled water (DW) resulted in the lowest values at all times when compared to all the other solutions. The value of the group stored in saline solution (S) for one month was below the other storage times compared among the groups stored in the same solution. In the groups stored in 0.5% chloramine-T (C), the value of the six-month storage was slightly below the others. The group stored in 0.1% thymol (T) for one month presented higher values than all the other groups (Table 3).

When compared to the control group (CG), the groups stored in DW for one week, one month, and six months presented statistical differences, showing lower values. Group T stored for one month presented higher values of bond strength to dentin than the CG. The other groups did not present significant statistical differences with the CG (Table 4).

4. Discussion

The storage of teeth for use in studies is a controversial issue and there is no consensus on the most appropriate treatment method for this purpose.[11] The results of the present study showed that both solution and time of teeth storage change the bond strength to dentin. These results reflect the structural change that dentin may suffer when stored in certain solutions at different times, for instance, a significant reduction in the levels of dentin minerals such as calcium, phosphorus, sodium, and potassium.[27]

In this study, regarding storage solutions, 0.5% chloramine-T resulted in the best results up to three months of storage. Although chloramine-T solution is an analogue to sodium hypochlorite, it does not affect collagen and apparently causes little changes in dental structures even at different concentrations, showing no significant influence on dentin up to three months.[2]

Thymol, in turn, is a solution commonly applied at various concentrations (0.02% to 0.1%) and times (24 hours to 6 months) in adhesion studies, because it presents antibacterial properties and consequently helps disinfecting teeth. However, there is controversy in the literature regarding the effect of this solution on the bond strength and structure of teeth, considering it presents mild acidity and may affect the dental content in long-term storage.[2] This may explain the effect of such solution in this study, which presented oscillations and instability of results at the storage times used, with higher bond strength values at one month, followed by six months, three months, and one week.

In disagreement with the results found, using 0.1% thymol for assessing bond strength for 24 hours, 15 days, one month, and six months with a self-etch adhesive of two steps showed no significant differences between the groups for the adhesive bond to dentin.[3] This difference may be caused by the type of adhesive system used in the dentin substrate, considering the present study used the conventional adhesive system of two steps, which has a greater decalcification potential of the dental substrate than self-etch adhesives, and the storage temperature and composite were different from the present study.

The 0.1% thymol and 0.5% chloramine-T solutions were also assessed for adhesive bond to dentin for three months using the same adhesive system of the present study and both solutions showed no decrease in adhesive bond to dentin, corroborating the results found in this study in the assessment for the same time.[16]

Differences are found in the literature for saline solutions as a means of tooth storage[29], considering these solutions would not be the most suitable for storing teeth,

because they may change the dentin structure and its permeability, likewise aqueous solutions such as distilled water.[14] Saline solution is an isotonic solution, which may cause an equilibrium of ions between the solution and the dentin substrate. This may explain the stability of such solution in the present study for the bond strength of teeth stored for up to six months, even though showing a slight decrease in the values presented for one month. Storage in distilled water resulted in the lowest values at all times when compared to all other solutions. This may occur due to the loss of mineral salts of the dentin substrate by osmosis, considering the solution is virtually free of these salts.

Regarding storage time, when dentin microhardness was assessed, it was not significantly affected at two months of storage, but it decreased statistically over 12 months, consequently affecting dentin adhesion, and thymol was one of the solutions that least affected dentin microhardness during this period.[1] Although the present study did not assess microhardness, this shows that storage time is an important factor to be considered, because it may produce different results in the same study, promoting ionic modifications between solution and dentin, in both intratubular collagen and glycoproteins. It may also cause changes in dentin permeability by intratubular ionic precipitation, affecting directly the results of bond strength and marginal infiltration.[17] The results of the present study also show the importance of standardizing storage time. Teeth stored in the same solution but at different times may present changes in the tests of bond strength to dentin. In this study, the three-month period was the only time in which none of the solutions presented statistically significant differences with the control group, indicating an appropriate period for storing teeth for dentin adhesion tests.

Regardless of the variation of results between the solutions and times, the result expected for a solution is to maintain most of the *in vivo* characteristics of the dental substrate. Therefore, the most suitable storage solutions and times to test the bond strength to dentine of bovine teeth at frozen temperatures are those without statistically significant difference with the group of teeth immediately extracted and not stored (control). Regarding the experimental groups, according to the present study, the only groups that presented statistical difference in relation to the control were distilled water at one week, one month, and six months and the group stored in 0.1% thymol for one month. Therefore, these solutions are not indicated for storing teeth for the adhesive bond test to dentine.

Another factor that may influence the bond strength to dentin and the results of the studies is the regional variation of the dentin substrate. Some *in vitro* studies compared the

strength values obtained at different sites of the tooth and confirmed such influence.[21] In the superficial dentin, the number of tubules is smaller than in deep dentin, and the penetration in the intertubular dentin depends on the degree of porosity that occurs in the collagen matrix due to acid etching. However, in deep dentin, the dentinal tubules are abundant and the intratubular permeability of the resin will be responsible for retention and sealing, affected by the difficulty in forming tags due to the presence of dentinal fluid and intratubular pressure. The orientation and density of the dentinal tubules may affect bond strength, which tends to be weaker when the number of tubules per area increases, and adhesives with prior acid etching are apparently more susceptible to regional differences than self-etch adhesives.[25] In this study, the specimens were produced in medium-depth dentin so that substrate depth would not interfere with the results and because of the great similarity between bovine and human teeth in this region.[3]

The rupture velocity of the load cell may also affect bond strength according to the study by Lindemuth and Hagge.[20] The authors used load cell velocities of 0.1, 0.5, 1, 5.0, and 10 mm/min and the results showed no statistically significant difference when bonding on enamel. However, the bond strength varied when bonding on dentin, presenting the lowest values for 0.1 and 10 mm/min and the highest values for 0.5, 1.0, and 5.0 mm/min.

Regarding storage temperature, when microleakage was assessed in deciduous teeth after storage, the numerical values suggested that the samples of the group at frozen temperatures allowed smaller marginal microleakage than the dry groups, which were stored dry for 30 days, hydrated, and stored in saline solution. This may be attributed to the fact that adhesives also react with the organic part of the dentin and perhaps this organic part may be preserved in better conditions through freezing.[13] Other studies also report better strength values when using freezing for tooth storage, indicating this temperature for dentin adhesion tests, especially when the location sites are in medium or deep dentin layers.[29]

Finally, one limitation of this study is that the period assessed was up to six months, and further studies should be performed over longer times with the same solutions, showing their effects on dentin substrate for longer periods.

5. Conclusion

According to the results obtained, it may be concluded that different storage solutions and times interfere with bond strength to dentin. Nonetheless, the groups stored in distilled water presented the lowest values and the groups in 0.5% chloramine-T presented the most stable values up to three months.

6. Tables

Table 1: Distribution of control and experimental groups (n = 8).

Storage solution	***Storage time***	***Storage temperature***
CG	-	-
DW	1 week, 1 month, 3 months, 6 months	-4ºC
S		
C		
T		

Table 2: Composition of the materials used.

Material	***Manufacturer***	***Composition***	***Classification***
Condac 37%	Dentscare LTDA, Joinville, SC, BRA	37% phosphoric acid, thickener, colorant and deionized water	acid conditioner
Adper Single Bond	3M ESPE, St Paul, MN, EUA	BisGMA, HEMA, dimethacrylates, ethanol, water, polyacrylic and polyalkanoic acid methacrylate functional copolymer, spherical silica particles with a diameter of 5 nanometers	two-step conventional adhesive system
Opallis Flow	*Dentscare LTDA, Joinville, SC, BRA*	*35% by weight of multifunctional methacrylate ester, 65% by weight of inorganic particles*	photopolymerizable composite

Source: Manufacturer information

Table 3 - Mean (SD) of bond strength to bovine dentin, as a function of time and storage solution.

Solution	***1 week***	***1 month***	***3 months***	***6 months***
DW	6.58(2.83)Bb	5.43(1.62)Cb	11.48(3.30)Ba	9.38(2.32)Ba
S	15.39(2.32)Aa	12.20(3.99)Bb	14.56(3.50)Aa	14.51(2.50)Aa
C	12.05(1.98)Aa	15.26(4.49)Ba	15.22(3.46)Aa	12.98(2.87)Aba
T	11.34(4.34)Ac	19.93(2.79)Aa	12.38(4.01)Bbc	14.93(2.52)Ab
CG: 14.08(3.83)				

Capital letters represent the values of the columns (times); lowercase represents the values of the rows (solutions).

Table 4 - Statistical comparison between control group and experimental groups.

Solution	***1 week***	***1 month***	***3 months***	***6 months***
DW	0,001*	0,001*	0,603	0,010*
S	0,570	0,352	0,800	0,794
C	0,204	0,582	0,544	0,528
T	0,201	0,004*	0,231	0,608

* 'p' value statistically different from the control group.

7. References

1. Aydin B, Pamir T, Baltaci A, Orman MN, Turk T. Effect of storage solutions on microhardness of crown enamel and dentin. Eur J Dent 2015; 9:262-266.

2. Boruziniat A, Danaifard N, Gifani M, Hakimi N. Effect of Storage Media and Sterilization Method on Shear Bond Strength of Composite to Enamel of Extracted Teeth. Dent Mater Tech. 2017; 6:96-102.

3. Cavalcanti AN, Santos-Daroz CB, Voltarelli FR, Lima AF, Peris AR, Marchi GM. Effect of the storage periods on the bond strength of a self-etch system to bovine dentin. Rev Odontol UNESP 2009; 38:222-227.

4. Chalipa J, Jalali YF, Gorjizadeh F, Baghaeian P, Hoseini MH, Mortezai O. Comparison of Bond Strength of Metal and Ceramic Brackets Bonded with Conventional and High-Power LED Light Curing Units. J Dent (Tehran) 2016; 13:423-430.

5. Dalpasquale G, Delbem ACB, Pessan JP, Nunes GP, Gorup LF, Neto FNS, De Camargo ER, Danelon M. Effect of the addition of nano-sized sodium hexametaphosphate to fluoride toothpastes on tooth demineralization: an in vitro study. Clin Oral Investig 2017; 21: 1821-1827.

6. De Araújo DF, Chaves LP, Bim O Jr, Pimentel Garcia FC, Ishikiriama SK, Honório HM, Wang L. Influence of 2% chlorhexidine digluconate on bond strength of a glass-fibre post luted with resin or glass-ionomer based cement. J Dent 2014; 42: 735-741.

7. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, Van Meerbeek B. Inhibition of enzymatic degradation of adhesive-dentin interfaces. J Dent Res 2009; 88:1101-1106.

8. Demenech LS, Tomazinho FSF, Tomazinho PH, Gabardo MCL, Baratto-Filho F. Avaliação de métodos de manutenção da esterilidade do órgão dental humano

extraído para armazenamento em banco de dentes. Revista da ABENO 2017; 17:55-65.

9. Di Hipólito V, Rodrigues FP, Piveta FB, Azevedo Lda C, Bruschi Alonso RC, Silikas N, Carvalho RM, De Goes MF, Perlatti D'Alpino PH. Effectiveness of self-adhesive luting cements in bonding to chlorhexidine-treaed dentin. Dent Mater 2012; 28:495-501.

10. Farret MM, Gonçalves TS, Lima SEM, Menezes LM, Oshima HMS, Kochenborger R, Freitas MPM. Influência de variáveis metodológicas na resistência de união ao cisalhamento. Dental Press J Orthod 2010; 15:80-88.

11. Freitas AR, Aznar ADC, Silva AL, Sales-Peres A, Sales-Peres SHC. Assessment of the effects of decontamination and storage methods on the structural integrity of human enamel. Rev Odontol UNESP 2016; 45:59-64.

12. Garcia RN, Souza CRS, Mazucco PEF, Justino LM, Schein MT, Giannini M. Avaliação da resistência de união de dois sistemas adesivos autocondicionantes – Revisão de literatura e aplicação do ensaio de microcisalhamento. RSBO 2007; 4: 37-45.

13. Ghersel ELA, Guedes-Pinto AC, Ciamponi AL. Influência do modo de armazenamento na microinfiltração de dentes decíduos restaurados com diferentes sistemas adesivos: estudo *in vitro*. Pesqui Odontol Bras 2001; 15:29-34.

14. Goodis HE, MarshalL GW, White JM, Gee L, Hornberger B, Marshall SJ. Storage effects on dentin permeability and shear bond strengths. Dental Mater 1993; 9:17-84.

15. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005; 84:741-746.

16. Humel MMC, Oliveira MT, Cavalli V, Giannini M. Effect of storage and disinfection methods of extracted bovine teeth on bond strength to dentin. Braz J Oral Sci 2007; 6:1402-1406.

17. Iório LS, Gomes APM, Kubo CH, Carneiro RGF, Carneiro BF, Silva EG. Avaliação da influência de diferentes meios de armazenamento de dentes humanos extraídos na infiltração marginal apical. Rev Odontol USP 2007; 19:173-180.

18. Kadowaki Y, Kakuda S, Kawano S, Katsumata A, Ting S, Hoshika S, Ikeda T, Tanaka T, Carvalho RM, Sano H. Bond performance of "Touch and Cure" adhesives on resin core systems. Dent Mater J 2016; 35:386-391.

19. Kimyai S, Pournaghi-Azar F, Mohammadi N, Babri M. Effect of hemostatic agent on marginal gaps of class V giomer restorations. J Clin Exp Dent 2017; 9:672-676.

20. Lindemuth JS, Hagge MS. Effect of Universal Testing Machine Crosshead Speed on the ShearNBond Strength and Bonding Failure Mode of Composite Resin to Enamel and Dentin. Military Medicine 2000; 165:742-746.

21. Lodovici E, Sadek FT, Muench A, Rodrigues Filho LE, Cardoso PEC. Influência do tempo de armazenamento na resistência de união à dentina de dois tipos de adesivos dentários por ensaio de microtração. Cienc Odontol Bras 2003; 6:79-86.

22. Lopes MB, Sinhoreti MAC, Sobrinho LC, Consani S. Comparative study of the dental substrate used in shear bond strength tests. Pesqui Odontol Bras 2003; 17: 171-175.

23. Neves TPC, Leandrin TP, Tonetto MR, Andrade MF, Campos EA. Resistência de união à microtração de sistemas adesivos “condiciona-e-lava” de dois passos: efeito de diferentes tratamentos da superfície dentinária condicionada. Rev Odontol UNESP 2017; 46:131-137.

24. Oliveira IK, Arsati YB, Basting RT, França FMG. Waiting time for coronal preparation and the influence of different cements on tensile strength of metal posts. International Journal of Dentistry 2012**;** 2012: 6 pages, Article ID 785427.

25. Pupo YM, Martins GC, Gomes GM, Michél MD, Gomes OMM, Gomes JC. Influência do tempo de armazenamento na resistência de união à microtração de diferentes sistemas adesivos em dentina superficial e profunda. Braz Dent Sci 2010; 13:16-22.

26. Rohr N, Fischer J. Tooth surface treatment strategies for adhesive cementation. J Adv Prosthodont 2017; 9:85-92.

27. Secilmis A, Dilber E, Gokmen F, Ozturk N, Telatar T. Effects of storage solutions on mineral contents of Dentin. Journal of Dental Sciences 2011; 6:189-194.

28. Silva L, Thedei G Jr, Menezes-Oliveira MA, Nogueira RD, Geraldo-Martins V. Tooth bleaching effects on the adhesive interface of composite restorations. Int J Esthet Dent 2017; 12:96-106.

29. Silva MF, Mandarino F, Sassi JF, Menezes M, Centola ALB, Nonaka T. Influência do tipo de armazenamento e do método de desinfecção de dentes extraídos sobre a adesão à estrutura dental. Rev Odontol USP 2006; 18:175-80.

30. Silva-Junior MF, Sousa ACC, Batista MJ, Sousa MLR. Condição de saúde bucal e motivos para extração dentária entre uma população de adultos (20-64 anos). Ciência & Saúde Coletiva 2017; 22:2693-2702.

31. Strawn SE, White JM, Marshall GW, Gee L, Goodis HE, Marshall SJ. Spectroscopic changes in human dentine exposed to various storage solutions – short term. J Dent 1996; 24:417‑423.

32. Wang L, Pinto TA, Silva LM, Araújo DF, Martins LM, Hannas AR, Pedreira AP, Francisconi PA, Honório HM. Effect of 2% chlorhexidine digluconate on bond strength of a glass-fibre post to root dentine. Int Endod J 2013; 9:847-854.

33. Xavier SR, Pilownic KJ, Gastmann AH, Echeverria MS, Romano AR, Pappen G. Bovine Tooth Discoloration Induced by Endodontic Filling Materials for Primary Teeth. Int J Dent 2017; 2017: 5 pages, Article ID 7401962.