Effects of 6-Month Water Storage on Microtensile Bond Strength of Universal Adhesives Applying in a Total-Etch Mode on Dentin

Suthatta Jeansuwannagorn* and Sirivimol Srisawasdi

Esthetic Restorative and Implant Dentistry Program, Faculty of Dentistry, Chulalongkorn University, Thailand
*Corresponding author, E-mail: Gag_SJ@hotmail.com

Abstract

This study aims to evaluate microtensile bond strength (µTBS) of universal adhesives applying in a total-etch mode after 24 hours and 6 months of water storage. Twenty-four extracted human third molars were cut to expose a flat dentin surface. Single Bond Universal (SU; 3M ESPE, USA), G-Premio BOND (GP; GC Corporation, Japan), and Prime&Bond Universal (PB; Dentsply, Germany) were used in total-etch mode following the manufacturers' instruction (n=8). After light-curing, composite resins were constructed on prepared dentin and then sectioned into 1×1×8 mm^3 slab to be tested after 24 hours and 6 months of water storage. The µTBS values of all universal adhesives were obtained analyzed with repeated measure ANOVA followed by Tukey Post Hoc test and Pair T-test (p=.05). The results revealed that µTBS of SU was not significantly different from PB (p=.286) but they were a statistically significant difference from GP (p<.001, p=.005, respectively). Considering the length of storage time, bond strength values of all adhesives applying in a total-etch mode were not statistically significantly decreased after 6 months of water storage comparing to 24 hours of water storage except G-Premio BOND (SU p=.1; GP p=.03; PB p=.814). In conclusion, universal adhesives, used in this study via a total-etch mode, performed differently. Water storage for 6 months affected bond strength of a HEMA-free universal adhesive, G-Premio BOND.

Keywords: *Bond strength test, Dentin, Microtensile bond strength, Total-etch, Universal Adhesive, Water storage*

1. Introduction

Establishing an effective bond between tooth substrates and resin composites remains a challenge in restorative dentistry (Irmak et al., 2016; Peumans et al., 2005). Type of the adhesive system has an important role in bonding to dentin and has an influence on the clinical performance of resin composite restoration.

A total-etch adhesive system that has been widely used, was proved to totally remove the smear layer of dental substrates via the action of phosphoric acid (Breschi et al., 2008; Perdigao, 2010; Tay & Pashley, 2002). Thus, this system had been shown to provide high-quality adhesion to both enamel and dentin (Van Meerbeek et al., 2003; Van Meerbeek et al., 2004). After conditioning with phosphoric acid to enamel, the hydroxyapatite (HAp) was created macro- and micro-porosities. This process allowed the resin to penetrate to form "prism-like" resin tags (Swift, Perdigao, & Heymann, 1995). On the other hand, the adhesion to dentin was more challenging due to the difference in enamel and dentin composition (Peumans et al., 2005). There was a few HAp left at the microporous network of collagen on the treated dentin surface after phosphoric acid application (Van Meerbeek et al., 2003). Moreover, water within the collagen network must be entirely replaced by resin monomers to achieve a stable bond (Breschi et al., 2018). One of the disadvantages of total-etch adhesive system was their susceptibility to variations in the degree of dentin moisture resulting in incomplete resin infiltration along the decalcified dentin (Dal-Bianco et al., 2006; Miyazaki, Onose, & Moore, 2002; Reis et al., 2007; Spencer & Swafford, 1999; Spencer et al., 2000). The phenomenon called interfacial hydrolytic degradation was considered to be the primary reason for resin degradation due to incomplete infiltration of resin monomers into the dentin collagen network of hybrid layer (Breschi et al., 2008; Frassetto et al., 2016; Sano, 2006). This phenomenon was a chemical reaction which broke covalent bonds affecting the loss of the resin mass (Breschi et al., 2008).

Current adhesive technology tends to simplify bonding procedures by reducing application steps, shortening clinical application time and decreasing technique sensitivity (Marchesi et al., 2014). Therefore, the new family of dental adhesives known as ''universal adhesive'' or ''multi-mode adhesive'' has been introduced. It was designed to bond to tooth structures via either total-etch technique or self-etch technique using the same single bottle of adhesive solution (Hanabusa et al., 2012; Rosa, Piva, & Silva, 2015; Wagner et al., 2014). For the total-etch technique, the application of an etching step prior to the universal adhesive application significantly improved their dentin penetration pattern when compared to the non-etching step (Wagner et al., 2014). From the previous study, it was shown that the dentin bond quality of universal adhesives did not have a negative effect when applied in a total-etch mode (Takamizawa et al., 2016). Jang et al. (2016) presented that the dentin bond strength of universal adhesive was comparable to contemporary multi-step adhesives.

Although there are several studies of enamel and dentin bonding performance using universal adhesives (Chen et al., 2015; Loguercio et al., 2015; McLean et al., 2015; Munoz et al., 2015; Wagner et al., 2014), only limited information is available on the bonding quality of universal adhesives using total-etch mode (Marchesi et al., 2014; Saikaew et al., 2016; Taschner et al., 2014). The purpose of this study was to evaluate microtensile bond strength (μTBS) of the universal adhesives applying in a total-etch mode after 24 hours and 6 months of water storage.

2. Objectives

The objective of this study was to evaluate the microtensile bond strength of universal adhesives applying in a total-etch mode after 24 hours and 6 months of water storage.

3. Materials and Methods

This study was approved by the ethical committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (approval number: HREC-DCU 2017-064).

Preparation of dentin specimens

Twenty-four extracted human third molars, free of debris and soft tissue, were stored in a 0.1% thymol solution at 4°C (Aydin et al., 2015). Teeth were analyzed following selection criteria: no caries nor previous restorations, no cracks. Teeth were mounted in a clear self-curing acrylic resin (Shofu, Japan) sized 22×18×12 mm^3 with the cementoenamel junction (CEJ) exposed (Figure 1A). Occlusal one-third of dental crowns were removed perpendicular to the long axis of each tooth using a water-cooled Isomet low-speed diamond saw (Isomet 1000, Buehler, USA) to expose a flat mid-coronal dentin surface (Figure 1B). Dentin surfaces were then treated by wet-sanding with a 600-grit silicon carbide sandpaper at 100 rpm for 30 s (Minitech 233, Presi, France) to produce standard smear layer (Finger, 1988; Pashley et al., 1988; Tao, Pashely, & Boyd, 1988).

Bonding and restorative procedure

The teeth were randomly assigned into 3 treatment groups (n = 8 per group, calculated from the pilot study) treated by three universal adhesives including Single Bond Universal (SU), G-Premio BOND (GP), Prime&Bond Universal (PB). The materials, components, and application methods, shown in Table 1 and 2, were used following manufacturers’ instructions.

Table 1: Materials, Manufacturers, and Components

Material, Manufacturer (batch number)	Components
Single Bond Universal Adhesive (3M ESPE, USA) Lot No. 651936	Adhesive: 10-MDP, Bis-GMA, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane-treated silica
G-Premio BOND (GC Corporation, Japan) Lot No. 1611211	Adhesive: 10-MDP, 4-MET, 10-MDTP, methacrylate acid ester, distilled water, acetone, photo initiators, silica fine powder
Prime&Bond Universal (Dentsply, Germany) Lot No. 1705000051	Adhesive: PENTA, 10-MDP, Bis-GMA, UDMA, TEGDMA, HEMA, Initiator, Stabilizer, Isopropanol, water
Premise (Kerr, USA) Lot No. 6093672	Filler: Prepolymerized filler (PPF),30-50 μm; Barium glass, 0.4 μm; Silica filler 0.02 μm Resin: Ethoxylated bis-phenol-A-dimethacrylate, TEGDMA, Light-cure initiators and stabilizers

HEMA: Hydroxyethyl methacrylate; Bis-GMA: Bisphenol A-glycidyl methacrylate 2,2-bis [4-(2-hydroxy-3-methacryloxypropoxy) phenyl] propane; MDP; Methacryloyloxydecyl dihydrogen phosphate; TEGDMA: Triethyleneglycol dimethacrylate; UDMA: Urethane dimethacrylate; MDTP: Methacryloyloxydecyl dihydrogen thiophosphate; PENTA: Phosphoric acid modified acrylate resin

Table 2: Material Application technique

Material	Manufacturer's instructions (total-etch mode)
Single Bond Universal Adhesive (SU)	1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets 2. Apply the adhesive to etched dentin, 5 µl, and rub it in for 20 s. 3. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 4. Light cure for 10 s.
G-Premio BOND (GP)	1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 2. Apply adhesive, 5 µl, and leave undisturbed for 10 s after application 3. Dry thoroughly for 5 s with oil free air under maximum air pressure 4. Light cure for 10 s
Prime&Bond Universal (PB)	1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 2. Adhesive applied to etch dentin, 5 µl, (do not desiccate) with rubbing action for 20 s. 3. Gentle stream of air applied over the liquid for at least 5 s. 4. Light cure for 10s

Following bonding procedures, in all groups, resin composite (Premise, shade XL1, Kerr, USA) was incrementally built up to a height of 4 mm (2 mm thick in each layer) (Figure 1C). Each increment was light-cured for 40 s from the top, and for 20 s at other surfaces, with light tip held perpendicularly and within 1 mm superior to resin composite. After the completion of the resin composite built up, all specimens were stored in water at 37°C for 24 hours.

Preparation of µTBS Specimens

All specimens were sectioned into 1×1×8 mm^3 bar-shape slabs using the Isomet low-speed diamond saw under water-cooling (Figure 1D). Eight slabs, using only the central dentin portion, were chosen and labeled (Eckert & Platt, 2007). All slabs were examined using a stereomicroscope (SZ61, Olympus, Japan) at 25× magnification to verify that structurally crack-free, intact slab and their exact dimensions were measured at a magnification of 40×. The sectioned slabs of each specimen were divided equally into 2 subgroups to store in distilled water at 37°C for 24 hours and 6 months.

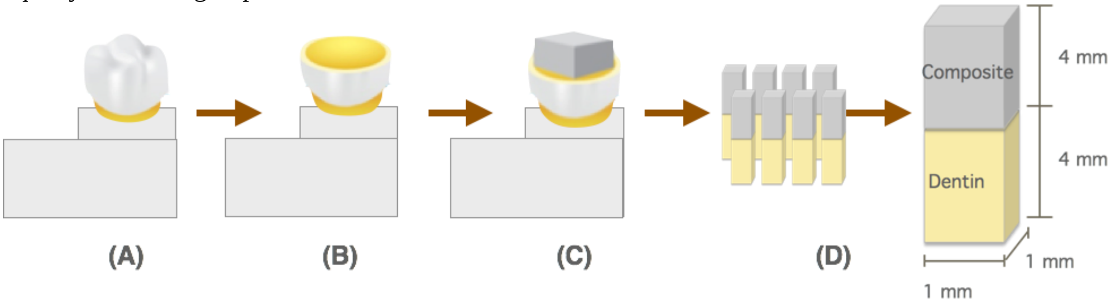

Figure 1 Preparation of Specimens

After storage, four sectioned slabs of each subgroups were attached to a Ciucchi's jig using a cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Japan) and subjected to µTBS testing using a universal testing machine (EZ-S; Shimadzu Corporation, Japan) at a cross-head speed of 1 mm/min (De Munck et al., 2009).

Statistical analysis

The µTBS values in MPa were recorded when the fracture occurred and analyzed statistically using SPSS 20.0 software (SPSS Inc., USA). The normality of the data was determined using the Shapiro-Wilk test. Repeated measure ANOVA and Tukey Post Hoc multiple comparison tests were used to determine the different of universal adhesives. Moreover, the storage period of universal adhesives was analyzed with Pair T-test. The level of significance was determined as p=.05.

Modes of failure

After debonding, the specimens were examined, under a stereomicroscope (SZ61, Olympus, Japan) at a magnification of 40×, to verify failure type. Failure modes were classified as shown in Table 3.

Table 3 Mode of failure

Mode of failure	
Co-De	Cohesive failure exclusively within dentin (>75% of the failure is within the dentin)
Co-Re	Cohesive failure exclusively within resin composite (>75% of the failure is within the resin composite)
Ad/Mixed	Adhesive failure at the resin/dentin interface (>75% of failure between resin/dentin interface included cohesive failure of the neighboring substrates)

4. Results and Discussion

The µTBS values were of a normal distribution (p>.05 in all groups). The µTBS means and standard deviations (SD) of 24-hour vs 6-month specimens for all adhesives are shown in Table 4.

The µTBS of the Single Bond Universal was not significantly different from Prime&Bond Universal (p=.286) but they were a statistically significant difference from G-Premio BOND (p<.001, p=.005, respectively). Considering the length of storage time, bond strength values of all adhesives applying in a total-etch mode were not statistically significantly decreased after 6 months of water storage specimen comparing to 24 hours of water storage except G-Premio BOND (SU p=.1; GP p=.03; PB p=.814).

Table 4 24-hour and 6-month water storage µTBS values (means ± SD (MPa)) of the universal adhesives

Adhesive	24-hour water storage	6-month water storage
SU	52.549 ± 12.344 [A, 1]	44.801 ± 17.425 [a, 1]
GP	25.948 ± 5.264 [B]	18.073 ± 6.736 [b]
PB	41.041 ± 13.295 [A, 1]	40.001 ± 11.191 [a, 1]

*Same capital letter means no statistically significant difference at 24-hour water storage; same lower case means no statistically significant difference at 6-month water storage; within the adhesive, same number means no statistically significant difference (*p*<.05)

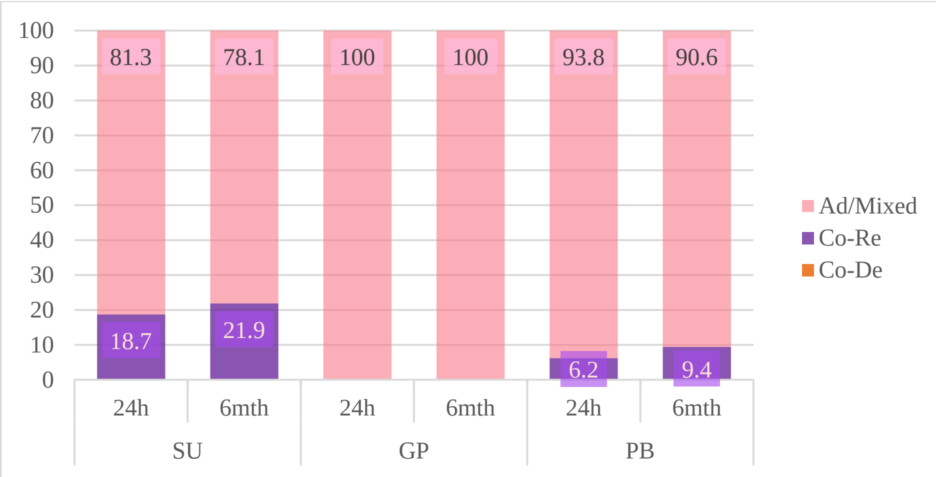

Figure 3 Failure mode analysis of universal adhesives bonded to dentin at 24 hours and 6 months of water storage

Failure type frequencies were given by group in Figure 3. Adhesive failure was noticed to be a major finding in all testing groups. Cohesive failure in resin composite was found in Single Bond Universal more than any other groups.

A new dental adhesive known as ‘‘universal adhesive’’ or ‘‘multi-mode adhesive” was developed and became more popular. This type of adhesive could be used with a wide range of substrates such as tooth structure, ceramic, and metal (Kim et al., 2015; Takamizawa et al., 2016). The universal adhesives were recommended by manufacturers to be used with etching or non-etching pretreatment to the dental structure. Many studies reported that the performance of bond strength and clinical appearance did not depend on the etching procedure (Burke et al., 2017; Forgerini et al., 2017; Rosa et al., 2015).

Universal adhesives contain multiple functional monomers, i.e. 10-MDP in all universal adhesive; 4-MET, 10-MDPT in G-Premio BOND; PENTA, UDMA, TEGDMA in Prime&Bond Universal. According to some former studies, 10-MDP created a chemical bond with hydroxyapatite crystals and turned into a stable calcium-phosphate salt (Inoue et al., 2005; Reis et al., 2009; Toledano et al., 2007; Waidyasekera et al., 2009; Yoshihara et al., 2011). The chemical bonding of 10-MDP improved bond strength and adhesion durability (Feitosa et al., 2012; Yoshida et al., 2012; Yoshihara et al., 2011). In addition, 4-MET also chemically bonded to hydroxyapatite. However, the chemical bonding ability of 4-MET was weaker than 10-MDP, and the solubility of calcium salt formed by 4-MET could be higher (Yoshida et al., 2004). These were the reasons why G-Premio BOND had the lowest µTBS in this study. This was in agreement with the previous study that 10-MDP-based adhesive had higher dentin µTBS as shown in the 4-MET-based adhesive (Inoue et al., 2003).

Moreover, the application method of universal adhesives was one of the factors which had an influence on dentin µTBS. G-Premio BOND was the only material that the manufacturer recommended to apply and leave the material undisturbed, whereas other adhesives were recommended to apply with a scrub or rub motion (Hanabusa et al., 2012; Munoz et al., 2014; Sezinando et al., 2015). The solvent evaporation and monomer infiltration occurred under active application could be increased. The active application also enhanced chemical interaction with the dentin substrate and improved polymerization efficacy. These led to an increase in dentin bond strength (Wang & Spencer, 2003; Zhang & Wang, 2012).

The μTBS of the adhesive-dentin bond of G-Premio BOND in this study after water storage for 6 months were significantly decreased when compared to water storage for 24 hours. However, the bond strengths of Single Bond Universal and Prime&Bond Universal adhesives were no significant decreased between 24 hours and 6 months of water storage. Similar to the previous study which demonstrated that the bond strength to dentin after 6 months aging in artificial saliva was reduced the bond strength of Single Bond Universal (irrespective of the application mode) compared to immediate values (Marchesi et al., 2014). The finding was consistent with others study, which indicated that the bond strength of Single Bond Universal when used in self-etch mode and total-etch mode was stable after 6 months of water storage (Munoz et al., 2015). The *in vitro* performance of universal adhesives has been reported to be material-dependent due to the complexity of their chemical composition (Hanabusa et al., 2012; Munoz et al., 2013). If the exposed collagen is not fully encapsulated by the polymerized adhesive monomers, demineralized collagen fibrils will be vulnerable to time-dependent hydrolytic degradation by water, leaving voids within the hybrid layer or demineralized nanochannels (Sano et al., 1994). The previous study found that voids within the bonding interface of G-Premio BOND were observed due to the manufacturer recommended the application, but not found in Single Bond Universal (Saikaew et al., 2018). Moreover, G-Premio BOND, a HEMA-free adhesive, was prone to phase separation affecting lower bonding quality (Van Landuyt et al., 2005).

Within the limitations of this study, only 24-hours compared to 6-month water storage bond strength values of a few universal adhesives were revealed. Thus, further study should be done focusing on improving bonding performance of adhesive systems using various of application techniques and comparing the bond strength to the other adhesives, such as the conventional total-etch adhesive and self-etch adhesive systems.

5. Conclusion

Universal adhesives that used in this study via a total-etch mode performed differently. The 6-month water storage affected bond strength of a HEMA-free universal adhesive, G-Premio BOND.

6. Acknowledgements

The authors declare no conflict of interest in this study. We would like to thank all the manufacturers that provided us with the adhesives used in the present study.

7. References

Aydin, B., Pamir, T., Baltaci, A., Orman, M. N., & Turk, T. (2015). Effect of storage solutions on microhardness of crown enamel and dentin. *European Journal of Dentistry, 9*(2), 262-266. doi:10.4103/1305-7456.156848

Breschi, L., Maravic, T., Cunha, S. R., Comba, A., Cadenaro, M., Tjaderhane, L., ... & Mazzoni, A. (2018). Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. *Dental Materials, 34*(1), 78-96. doi:10.1016/j.dental.2017.11.005

Breschi, L., Mazzoni, A., Ruggeri, A., Cadenaro, M., Di Lenarda, R., & De Stefano Dorigo, E. (2008). Dental adhesion review: aging and stability of the bonded interface. *Dental Materials, 24*(1), 90-101. doi:10.1016/j.dental.2007.02.009

Burke, F. J. T., Crisp, R. J., Cowan, A. J., Raybould, L., Redfearn, P., Sands, P., ... & Ravaghi, V. (2017). A Randomised Controlled Trial of a Universal Bonding Agent at Three Years: Self Etch vs Total Etch. *European Journal of Prosthodontics and Restorative Dentistry, 25*(4), 220-227. doi:10.1922/EJPRD_01692Burke08

Chen, C., Niu, L. N., Xie, H., Zhang, Z. Y., Zhou, L. Q., Jiao, K., ... & Tay, F. R. (2015). Bonding of universal adhesives to dentine--Old wine in new bottles? *Journal of Dentistry, 43*(5), 525-536. doi:10.1016/j.jdent.2015.03.004

Dal-Bianco, K., Pellizzaro, A., Patzlaft, R., de Oliveira Bauer, J. R., Loguercio, A. D., & Reis, A. (2006). Effects of moisture degree and rubbing action on the immediate resin-dentin bond strength. *Dental Materials, 22*(12), 1150-1156. doi:10.1016/j.dental.2005.10.010

De Munck, J., Van den Steen, P. E., Mine, A., Van Landuyt, K. L., Poitevin, A., Opdenakker, G., & Van Meerbeek, B. (2009). Inhibition of enzymatic degradation of adhesive-dentin interfaces. *Journal of Dental Research, 88*(12), 1101-1106. doi:10.1177/0022034509346952

Eckert, G. J., & Platt, J. A. (2007). A statistical evaluation of microtensile bond strength methodology for dental adhesives. *Dental Materials, 23*(3), 385-391. doi:10.1016/j.dental.2006.02.007

Feitosa, V. P., Leme, A. A., Sauro, S., Correr-Sobrinho, L., Watson, T. F., Sinhoreti, M. A., & Correr, A. B. (2012). Hydrolytic degradation of the resin-dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing. *Journal of Dentistry, 40*(12), 1134-1143. doi:10.1016/j.jdent.2012.09.011

Finger, W. J. (1988). Dentin bonding agents. Relevance of in vitro investigations. *American Journal of Dentistry, 1 Spec No*, 184-188.

Forgerini, T. V., Ribeiro, J. F., Rocha, R. O., Soares, F. Z., & Lenzi, T. L. (2017). Role of Etching Mode on Bonding Longevity of a Universal Adhesive to Eroded Dentin. *Journal of Adhesive Dentistry, 19*(1), 69-75. doi:10.3290/j.jad.a37723

Frassetto, A., Breschi, L., Turco, G., Marchesi, G., Di Lenarda, R., Tay, F. R., ... & Cadenaro, M. (2016). Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability--A literature review. *Dental Materials, 32*(2), e41-53. doi:10.1016/j.dental.2015.11.007

Hanabusa, M., Mine, A., Kuboki, T., Momoi, Y., Van Ende, A., Van Meerbeek, B., & De Munck, J. (2012). Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. *Journal of Dentistry, 40*(6), 475-484. doi:10.1016/j.jdent.2012.02.012

Inoue, S., Koshiro, K., Yoshida, Y., De Munck, J., Nagakane, K., Suzuki, K., … & Van Meerbeek, B. (2005). Hydrolytic stability of self-etch adhesives bonded to dentin. *Journal of Dental Research, 84*(12), 1160-1164. doi:10.1177/154405910508401213

Inoue, S., Vargas, M. A., Abe, Y., Yoshida, Y., Lambrechts, P., Vanherle, G., ... & Van Meerbeek, B. (2003). Microtensile bond strength of eleven contemporary adhesives to enamel. *American Journal of Dentistry, 16*(5), 329-334.

Irmak, O., Baltacioglu, I. H., Ulusoy, N., & Bagis, Y. H. (2016). Solvent type influences bond strength to air or blot-dried dentin. *BMC Oral Health, 16*(1), 77. doi:10.1186/s12903-016-0247-3

Jang, J. H., Lee, M. G., Woo, S. U., Lee, C. O., Yi, J. K., & Kim, D. S. (2016). Comparative study of the dentin bond strength of a new universal adhesive. *Dental Materials Journal, 35*(4), 606-612. doi:10.4012/dmj.2015-422

Kim, J. H., Chae, S. Y., Lee, Y., Han, G. J., & Cho, B. H. (2015). Effects of multipurpose, universal adhesives on resin bonding to zirconia ceramic. *Operative Dentistry, 40*(1), 55-62. doi:10.2341/13-303-L

Loguercio, A. D., de Paula, E. A., Hass, V., Luque-Martinez, I., Reis, A., & Perdigao, J. (2015). A new universal simplified adhesive: 36-Month randomized double-blind clinical trial. *Journal of Dentistry, 43*(9), 1083-1092. doi:10.1016/j.jdent.2015.07.005

Marchesi, G., Frassetto, A., Mazzoni, A., Apolonio, F., Diolosa, M., Cadenaro, M., ... & Breschi, L. (2014). Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. *Journal of Dentistry, 42*(5), 603-612. doi:10.1016/j.jdent.2013.12.008

McLean, D. E., Meyers, E. J., Guillory, V. L., & Vandewalle, K. S. (2015). Enamel Bond Strength of New Universal Adhesive Bonding Agents. *Operative Dentistry, 40*(4), 410-417. doi:10.2341/13-287-L

Miyazaki, M., Onose, H., & Moore, B. K. (2002). Analysis of the dentin-resin interface by use of laser Raman spectroscopy. *Dental Materials, 18*(8), 576-580.

Munoz, M. A., Luque, I., Hass, V., Reis, A., Loguercio, A. D., & Bombarda, N. H. (2013). Immediate bonding properties of universal adhesives to dentine. *Journal of Dentistry, 41*(5), 404-411. doi:10.1016/j.jdent.2013.03.001

Munoz, M. A., Luque-Martinez, I., Malaquias, P., Hass, V., Reis, A., Campanha, N. H., & Loguercio, A. D. (2015). In vitro longevity of bonding properties of universal adhesives to dentin. *Operative Dentistry, 40*(3), 282-292. doi:10.2341/14-055-L

Munoz, M. A., Sezinando, A., Luque-Martinez, I., Szesz, A. L., Reis, A., Loguercio, A. D., ... & Perdigao, J. (2014). Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. *Journal of Dentistry, 42*(5), 595-602. doi:10.1016/j.jdent.2014.01.013

Pashley, D. H., Tao, L., Boyd, L., King, G. E., & Horner, J. A. (1988). Scanning electron microscopy of the substructure of smear layers in human dentine. *Archives of Oral Biology, 33*(4), 265-270.

Perdigao, J. (2010). Dentin bonding-variables related to the clinical situation and the substrate treatment. *Dental Materials, 26*(2), e24-37. doi:10.1016/j.dental.2009.11.149

Peumans, M., Kanumilli, P., De Munck, J., Van Landuyt, K., Lambrechts, P., & Van Meerbeek, B. (2005). Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. *Dental Materials, 21*(9), 864-881. doi:10.1016/j.dental.2005.02.003

Reis, A., Leite, T. M., Matte, K., Michels, R., Amaral, R. C., Geraldeli, S., & Loguercio, A. D. (2009). Improving clinical retention of one-step self-etching adhesive systems with an additional hydrophobic adhesive layer. *Journal of the American Dental Association, 140*(7), 877-885.

Reis, A., Pellizzaro, A., Dal-Bianco, K., Gones, O. M., Patzlaff, R., & Loguercio, A. D. (2007). Impact of adhesive application to wet and dry dentin on long-term resin-dentin bond strengths. *Operative Dentistry, 32*(4), 380-387. doi:10.2341/06-107

Rosa, W. L., Piva, E., & Silva, A. F. (2015). Bond strength of universal adhesives: A systematic review and meta-analysis. *Journal of Dentistry, 43*(7), 765-776. doi:10.1016/j.jdent.2015.04.003

Saikaew, P., Chowdhury, A. F., Fukuyama, M., Kakuda, S., Carvalho, R. M., & Sano, H. (2016). The effect of dentine surface preparation and reduced application time of adhesive on bonding strength. *Journal of Dentistry, 47*, 63-70. doi:10.1016/j.jdent.2016.02.001

Saikaew, P., Fu, J., Chowdhury, A., Carvalho, R. M., & Sano, H. (2018). Effect of air-blowing time and long-term storage on bond strength of universal adhesives to dentin. *Clinical Oral Investigations*. doi:10.1007/s00784-018-2656-y

Sano, H. (2006). Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds. *Journal of Dental Research, 85*(1), 11-14. doi:10.1177/154405910608500102

Sano, H., Shono, T., Takatsu, T., & Hosoda, H. (1994). Microporous dentin zone beneath resin-impregnated layer. *Operative Dentistry, 19*(2), 59-64.

Sezinando, A., Luque-Martinez, I., Munoz, M. A., Reis, A., Loguercio, A. D., & Perdigao, J. (2015). Influence of a hydrophobic resin coating on the immediate and 6-month dentin bonding of three universal adhesives. *Dental Materials, 31*(10), e236-246.

Spencer, P., & Swafford, J. R. (1999). Unprotected protein at the dentin-adhesive interface. *Quintessence International, 30*(7), 501-507.

Spencer, P., Wang, Y., Walker, M. P., Wieliczka, D. M., & Swafford, J. R. (2000). Interfacial chemistry of the dentin/adhesive bond. *Journal of Dental Research, 79*(7), 1458-1463. doi:10.1177/00220345000790070501

Swift, E. J., Jr., Perdigao, J., & Heymann, H. O. (1995). Bonding to enamel and dentin: a brief history and state of the art, 1995. *Quintessence International, 26*(2), 95-110.

Takamizawa, T., Barkmeier, W. W., Tsujimoto, A., Berry, T. P., Watanabe, H., Erickson, R. L., ... & Miyazaki, M. (2016). Influence of different etching modes on bond strength and fatigue strength to dentin using universal adhesive systems. *Dental Materials, 32*(2), E9-E21. doi:10.1016/j.dental.7015.11.005

Tao, L., Pashely, D. H., & Boyd, L. (1988). Effect of different types of smear layers on dentin and enamel shear bond strengths. *Dental Materials, 4*(4), 208-216.

Taschner, M., Kummerling, M., Lohbauer, U., Breschi, L., Petschelt, A., & Frankenberger, R. (2014). Effect of double-layer application on dentin bond durability of one-step self-etch adhesives. *Operative Dentistry, 39*(4), 416-426. doi:10.2341/13-168-L

Tay, F. R., & Pashley, D. H. (2002). Dental adhesives of the future. *Journal of Adhesive Dentistry, 4*(2), 91-103.

Toledano, M., Osorio, R., Osorio, E., Aguilera, F. S., Yamauti, M., Pashley, D. H., & Tay, F. (2007). Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media. *Dental Materials, 23*(7), 885-892. doi:10.1016/j.dental.2006.06.030

Van Landuyt, K. L., De Munck, J., Snauwaert, J., Coutinho, E., Poitevin, A., Yoshida, Y., ... & Van Meerbeek, B. (2005). Monomer-solvent phase separation in one-step self-etch adhesives. *Journal of Dental Research, 84*(2), 183-188. doi:10.1177/154405910508400214

Van Meerbeek, B., De Munck, J., Yoshida, Y., Inoue, S., Vargas, M., Vijay, P., ... & Vanherle, G. (2003). Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Operative Dentistry, 28*(3), 215-235.

Van Meerbeek, B., Kanumilli, P. V., De Munck, J., Van Landuyt, K., Lambrechts, P., & Peumans, M. (2004). A randomized, controlled trial evaluating the three-year clinical effectiveness of two etch & rinse adhesives in cervical lesions. *Operative Dentistry, 29*(4), 376-385.

Wagner, A., Wendler, M., Petschelt, A., Belli, R., & Lohbauer, U. (2014). Bonding performance of universal adhesives in different etching modes. *Journal of Dentistry, 42*(7), 800-807. doi:10.1016/j.jdent.2014.04.012

Waidyasekera, K., Nikaido, T., Weerasinghe, D. S., Ichinose, S., & Tagami, J. (2009). Reinforcement of dentin in self-etch adhesive technology: a new concept. *Journal of Dentistry, 37*(8), 604-609. doi:10.1016/j.jdent.2009.03.021

Wang, Y., & Spencer, P. (2003). Hybridization efficiency of the adhesive/dentin interface with wet bonding. *Journal of Dental Research, 82*(2), 141-145. doi:10.1177/154405910308200213

Yoshida, Y., Nagakane, K., Fukuda, R., Nakayama, Y., Okazaki, M., Shintani, H., ... & Van Meerbeek, B. (2004). Comparative study on adhesive performance of functional monomers. *Journal of Dental Research, 83*(6), 454-458. doi:10.1177/154405910408300604

Yoshida, Y., Yoshihara, K., Nagaoka, N., Hayakawa, S., Torii, Y., Ogawa, T., ... & Van Meerbeek, B. (2012). Self-assembled Nano-layering at the Adhesive Interface. *Journal of Dental Research, 91*(4), 376-381. doi:10.1177/0022034512437375

Yoshihara, K., Yoshida, Y., Hayakawa, S., Nagaoka, N., Irie, M., Ogawa, T., ... & Van Meerbeek, B. (2011). Nanolayering of phosphoric acid ester monomer on enamel and dentin. *Acta Biomaterialia, 7*(8), 3187-3195. doi:10.1016/j.actbio.2011.04.026

Zhang, Y., & Wang, Y. (2012). Distinct photopolymerization efficacy on dentin of self-etch adhesives. *Journal of Dental Research, 91*(8), 795-799. doi:10.1177/0022034512452143