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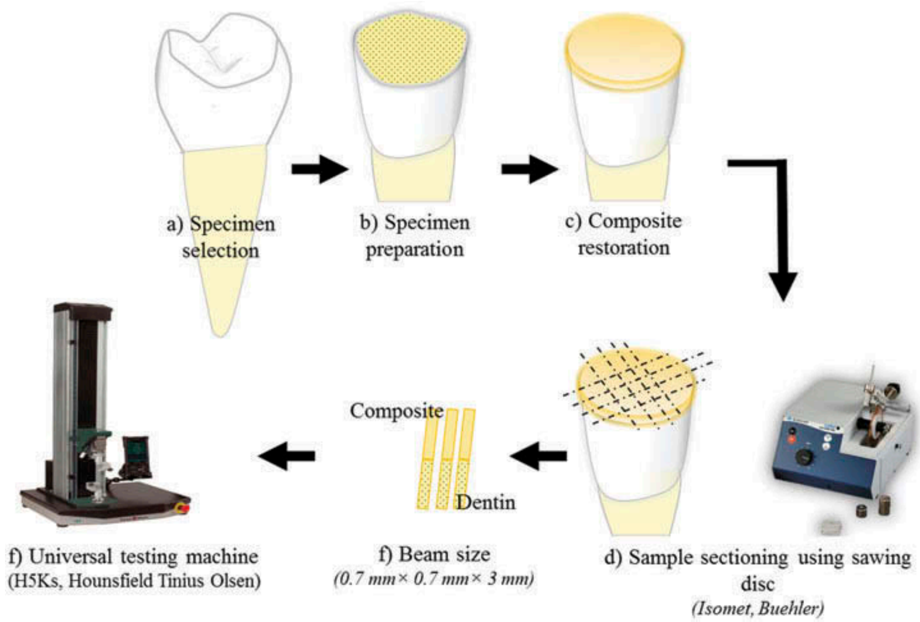

BIOMECHANICS | RESEARCH ARTICLE

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Cogent Engineering (2018), 5: 1472052

Bakhsh et al., *Cogent Engineering* (2018), 5: 1472052
https://doi.org/10.1080/23311916.2018.1472052

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Time sensitivity associated with the application of water-based all-in-one adhesive system

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Received: 13 February 2018
Accepted: 26 April 2018
First Published: 29 May 2018

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Reviewing editor:
Zhongmin Jin, Xian Jiao Tong University (China) and Leeds University (UK), China

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Abstract: This *in vitro* study was performed to investigate effect of delayed composite application on dentin bonding durability of water-based all-in-one self-etch adhesive system using micro-tensile bond strength (MTBS) testing. Fifteen freshly extracted, non-carious premolar teeth were randomly selected. After removing the roots, the teeth were occlusally trimmed to expose superficial dentin. Then, the exposed occlusal dentin was bonded with a self-etching adhesive (Tetric-N Bond self-etch; Ivoclar/Vivdent) according to the manufacturer's instructions. After that, the samples were divided equally into 3 groups based on the time of application of Tetric-N Ceram composite resin (Ivoclar/Vivdent, Liechtenstein). In group 1, the composite build-up was carried out immediately after curing the adhesive, and polymerized according to the manufacturer instructions. In groups 2 and 3, the samples were restored after 1 and 5 min following adhesive curing, respectively. After 24 h storage, each bonded sample was sectioned into beams (0.7 mm × 0.7 mm) for MTBS testing. Results of the MTBS were analysed and tested using one-way ANOVA and post hoc Tukey test at significance level of 5%. The results showed that applying composite restoration immediately after adhesive curing gave strength of 11 ± 6 MPa. Composite application after curing the adhesive by 1 and 5 min showed 16 ± 8 MPa and 11 ± 5 MPa, respectively. The 1 min delayed application group had significantly higher bond strength than the other tested groups ($p > 0.05$). In conclusion, delaying the composite application after polymerization of water-based all-in-one adhesive for 1 min showed better bond strength.

Subjects: Biomaterials; Biomechanics; Biomaterials and Medical Devices; Composites; Dentistry

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Dr. Turki Bakhsh - B.D.S.- King Abdulaziz University (2013) - CACT, PhD - Tokyo Medical and Dental University (2013). Turki Bakhsh received his PhD in 2D/3D Biomedical Imaging of nano-technology based dental composites from Tokyo Medical and Dental University, Tokyo, Japan. Currently, he is an associate professor at King AbdulAziz University, Saudi Arabia. His main area of interest in research are composite adaptation and characterization of the nano-interaction zone at tooth-resin interface using Optical Coherence Tomography, Micro Computed Tomography, Focused Ion Beam/TEM-SEM and CLSM.

PUBLIC INTEREST STATEMENT

With the advancement in adhesive dentistry, it is difficult for clinicians to predict the long-term clinical performance of the currently available dental bonding systems. Although many investigators believe water-based dental adhesives have a great potential and can bond effectively to the viable dentin, no study had tested the time sensitivity associated with its application technique. This study showed that delaying composite restoration up to 1 min might optimize the bond strength of water-based adhesives.

Keywords: adhesion; adhesive; all-in-one adhesive; dentin; micro-tensile bond strength; SEM; dental; tooth; biomechanics

1. Background

Nanoscience is the science of imaging and manipulating materials at nano-scale (Abiodun-Solanke, Ajayi, & Arigbede, 2014; Sadeghpour, Amirjani, Hafezi, & Zamanian, 2014). As this technology has a great potential, it has attracted marked attention in medical and dental field that are concerned with nanostructures and nanomaterials at less than 100 nm (Abou Neel, Bozec, Perez, Kim, & Knowles, 2015). Some of these nanomaterials are in a form of crystalline or particles that have been extensively used by the manufacturers to enhance the mechanical and physical properties. This in return will improve the material's interaction with biological structures at nano- and micro-scale (Amirjani, Hafezi, Zamanian, Yasaee, & Osman, 2016; Sadeghpour et al., 2014).

Intimate adhesion between a dental adhesive and composite filling is one of the essentials for the success of the dental restoration (Van Landuyt et al., 2007). The future aim of adhesive dentistry is to be less dependent on macro-mechanical retention and more conservative on tooth preparation with less removal of unsupported enamel (Giannini et al., 2015).

Current development of dental bonding can be recognized in two approaches. The first approach is the etch and rinse adhesive system (i.e. total-etch system), which is known as the most effective approach in enamel bonding (Ozer & Blatz, 2013). This system starts with an acid etching step (commonly with a 30–40% phosphoric acid gel) to completely remove the smear layer and its plugs (Cardoso et al., 2011) through selective dissolution of hydroxyapatite crystals in enamel and exposure of micropores in the dentin collagen (Marghalani, Bakhsh, Sadr, & Tagami, 2014, 2015; Van Meerbeek, De Munck, Yoshida, et al., 2003). After etching, a separate primer or primer mixed with adhesive bonding dissolved in an organic solvent such as water, ethanol or acetone, is usually applied on the tooth surface before composite restoration (Cardoso et al., 2011).

The second bonding approach is the self-etch approach (i.e. self-etch adhesive system) (Ozer & Blatz, 2013) which does not require a separate etching step as its formula is composed of solvents and acidic functional monomers that can etch and prime the dental substrate at the same time (Bakhsh, Sadr, & Tagami, 2015; Cardoso et al., 2011; Van Meerbeek et al., 2003). This system is subdivided into a two-step self-etch adhesive and a one-step self-etch adhesive (also known as an all-in-one adhesive).

The self-etch approach is characterized by easy manipulation and reduced post-operative sensitivity as well as the risk of making technical errors during application, which explains the popularity of this adhesive system among dental practitioners as a common approach in dentin bonding (Bakhsh et al., 2015, 2013, 2015).

Many studies have investigated the effect of delaying composite activation, after all-in-one adhesive application, on dentin bonding strength (Asaka, Miyazaki, Takamizawa, Tsubota, & Moore, 2006; Tay, King, & Suh et al., 2001, Tay, Pashley, Suh, Carvalho, & Itthagarun, 2002). The dentin bond strengths of several all-in-one adhesive systems were reported to be affected by time (Asaka et al., 2006). A comparison between different adhesives showed an increase in bond strength when the application of the composite restoration was delayed up to 2 min regardless of the adhesive utilized (Asaka et al., 2006). Interestingly, the predominant failure in that study was adhesive failure in immediately restored groups. Whilst in delayed composite placement groups, the adhesive, composite and dentine failures prevailed (Asaka et al., 2006). Polymerization of composite was supposed to be affected by the acid-base reaction between tertiary amines and the acidic functional monomers in the adhesive, which ends with reduction of the polymerization rate (Tay, Pashley, & Yiu et al., 2003).

Since no study has investigated the effect of time on the bond strength of water-based HEMA-rich all-in-one dental adhesive, so far, this study was conducted to investigate it using micro-tensile bond strength (MTBS). The null hypothesis was that delaying composite application following water-based all-in-one adhesive curing has a negative effect on the dentin bond strength.

2. Materials and methods

2.1. Sample preparation

The experimental design implemented in this study complies with the guiding principles for experimental procedures found in the Declaration of Helsinki of the World Medical Association, and was duly cleared by the Institutional Review Board and institutional Ethics Committee of King Abdulaziz University.

Fifteen extracted human premolar teeth with no restorations or caries lesions were used in this study and stored in normal saline (0.9% w/v sodium chloride) prior to their usage. The teeth were cleaned from any remnants of calculus and inspected for any cracking or crazing that might be induced during dental extraction. The selected samples were randomly divided into three experimental groups according to the time of composite build-up.

The entire occlusal enamel was removed by trimming under continuous water cooling to obtain flat dentin surface, and the roots were removed by using a low-speed diamond saw (IsoMet 1000, Buehler, USA) under running water coolant. In order to form smear layer on the exposed dentin surface, the surface was polished with wet sandpaper (600 grit) for 30 s, then a self-etching adhesive (Tetric-N self-etch, Ivoclar/Vivdent, Liechtenstein) was applied on the exposed dentin surface according to the manufacturer's instructions to all groups (Table 1). Afterward, group 1 was restored immediately within 10 s after adhesive curing with Tetric-N Ceram composite resin (Ivoclar/Vivdent, Liechtenstein), which was applied in two increments, 2 mm thickness of each increment, and cured according to the manufacturer's instructions (Table 1). In group 2, the samples were restored after 1 min, while the samples in group 3 were restored 5 min after adhesive curing.

The bonded specimens were stored in distilled water at 37°C for 24 h. After that, they were cross-sectioned longitudinally in crisscross pattern with a low-speed diamond saw under continuous water-cooling to obtain multiple beam-shaped sticks (0.7 mm × 0.7 mm) from all groups. After cutting into beams, each specimen was tested with an MTBS testing machine. The total sample size was 40 in each group.

2.2. Micro-tensile bond strength test

The MTBS was tested with a universal testing machine (H5Ks, Hounsfield Tinius Olsen, UK) at crosshead speed of 1 mm/min. The bonded surface area of each resin-dentin specimen was approximately (0.7 × 0.7 mm^2) in dimension and was attached to specially designed metal plates by superglue (Model Repair II Blue, Dentsply-Sankin, Japan). Each beam was placed in the testing machine perpendicular to the floor, and the tensile load was derived by dividing the applied force (Newton) at the time of point of failure by resin-dentin bonded area (mm^2) and expressed in MPa. The load at point of failure (Newton) at the resin-dentin bonded area (mm^2) was determined. Later, imaging with a scanning electron microscope (SEM) was performed to verify the failure mode. Schematic illustration of the sample preparation and testing is described in Figure 1.

2.3. SEM observation

All specimens from three experimental groups (1, 2, 3) were prepared and subjected to SEM (Quanta, 250 SEM; FEI, USA) examination.

Table 1. Materials used in the study

Material (Manufacturer)	Composition	Lot no.	Application Mode
Tetric-N Bond Self-Etch (Ivoclar/Vivadent) pH = 1.5	• Bis-acrylamide • Bis-methacrylamide dihydrogen phosphate (HEMA) • Amino acid acrylamide • Hydroxy alkyl methacrylamide • highly dispersed silicon dioxide • Water (20–30 wt.%) • Catalysts • Stabilizers	S22554	(1) Active application of a thick layer of Tetric N-Bond Self- Etch on the enamel and dentin surfaces for at least 30 s. (2) Air-drying with a strong stream of air until there is no longer any movement of the material. (3) Light irradiation for 10 s at a light intensity of more than 500 mW/cm^2.
Tetric-N Ceram Composite (Ivoclar/Vivadent)	• Bisphenol A-glycidyl methacrylate • Urethane dimethacrylate • Decandiol dimethacrylate (19 wt.%). • Barium glass • Ba-Al-fluoro-silicate glass • Ytterbium trifluoride • Highly dispersed silicon dioxide • Steroid mixed oxide (81 wt.%) • Catalysts, stabilizers and pigments (0.8 wt.%) The total content of inorganic fillers is 81 wt.% (63 vol.%)	R00009 S38944 R62326	(1) Application of Tertic Ceram composite in 2 increments. (2) Light curing of each increment with a light-curing unit (>500 mW/cm^2) for 20 s.

After specimens drying at room temperature, they were sputter coated with a gold layer in a vacuum apparatus. Dentin surfaces of the fracture sites were observed under SEM with different magnifications (×85—×2,500) to classify the mode of failure. The mode of failure was categorized into; (1) adhesive failure; when the fracture site was entirely within the adhesive, (2) mixed failure; when the fracture site continued from the adhesive into either resin composite or dentin and (3) cohesive failure; when the fracture occurred exclusively within the resin composite or dentin.

2.4. Statistical analysis

All data were statistically analysed by the Statistical Package for the Social Sciences version 22 SPSS (IBM, Armonk, USA). Analysis of variance (ANOVA) was used to test the significant difference between continuous variables at 5% level of significance. A post hoc Tukey test was used for pairwise comparison.

3. Results

Composite application and curing immediately (Group 1) gave mean strength of 11 ± 6 MPa. After delaying composite application in groups 2 and 3, the mean MTBSs were 16 ± 8 MPa and 11 ± 5 MPa, respectively (Table 2).

One-way ANOVA testing indicated a strong significant difference in the mean adhesion strength between different adhesive timing protocols with p-value of 0.0001 (Table 3). A post hoc Tukey test

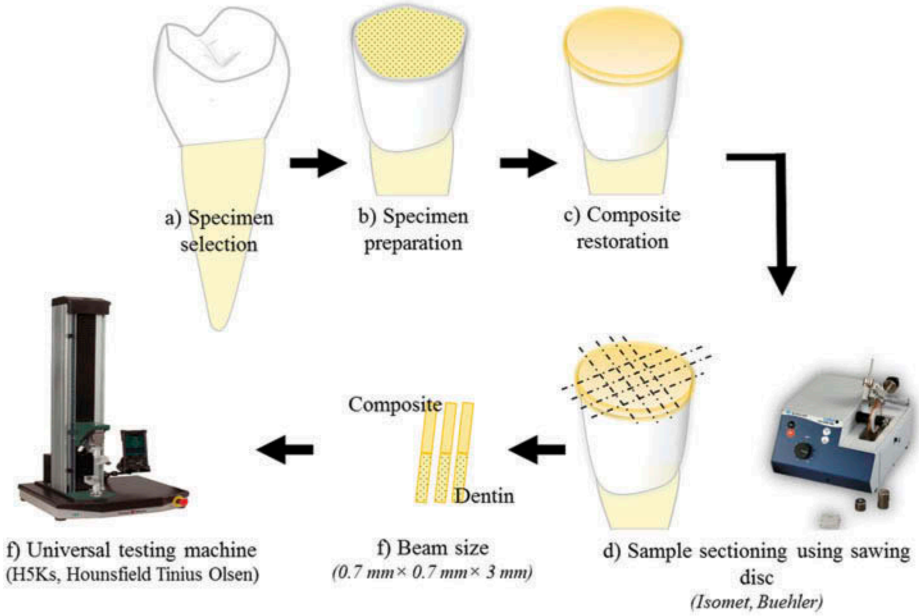

Figure 1. Schematic diagram showing the methodology of the study. After selection of the teeth, the occlusal surfaces were trimmed until the underlying superficial dentin was exposed. Then, the samples were divided into 3 groups according to the restoration protocol. Later, the bonded specimens were equally sectioned in crisscross pattern using slow-speed sawing machine to generate 3 mm length rectangular beams (width 0.7 mm × length 0.7 mm). All beams were subjected to micro-tensile bond strength testing using universal testing machine. MTBS: Micro-tensile bond strength test

showed that group 2 showed the highest mean adhesion strength followed by groups 1 and 3. Group 2 was significantly higher by 4.8 MPa and 5.3 MPa compared to groups 1 and 3, respectively. No significant difference between groups 1 and 3 (Table 4).

SEM images showed mixed failures between adhesive and dentin, and adhesive failures. None of the specimens showed cohesive failures in composite or dentin (Figure 2).

4. Discussion

This study investigated the effect of delayed composite application on the bond strength, which showed improved bond strength after 1 min.

Generally, homogenization between the hydrophilic with hydrophobic monomers in all-in-one adhesive takes place in the presence of the organic solvents (Giannini et al., 2015). When these solvents are in the form of ethanol or acetone, they also act as water chaser around dentin collagen and improve monomer impregnation into dentin (Giannini et al., 2015; Ozer & Blatz, 2013; Qari, Banakhar, & Aljafari et al., 2016). However, the solvent in some all-in-one adhesives

Table 2. Descriptive analyses of the bonding strength of three-bonding protocols in regards to timing

Bonding Protocol	N	Maximum	Minimum	Mean ± SD
Group 1	40	2.1	26.6	11 ± 6
Group 2	40	2.4	38.2	16 ± 8
Group 3	40	3.3	23.7	11 ± 5

Table 3. One-way ANOVA test to estimate the difference in mean bonding strength between three-bonding protocols in regards to timing

	Mean Square	F-statistics	p-Value
Between Groups	341.9	8.3	0.0001
Within Groups	41.3		

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Table 4. Post hoc Tukey test for multiple comparisons between groups of three bonding protocols in regards to timing

Groups Comparison		Mean Difference	*p*-Value
Group 1	Group 2	4.77	0.003
	Group 3	0.53	0.9
Group 2	Group 3	5.3	0.001

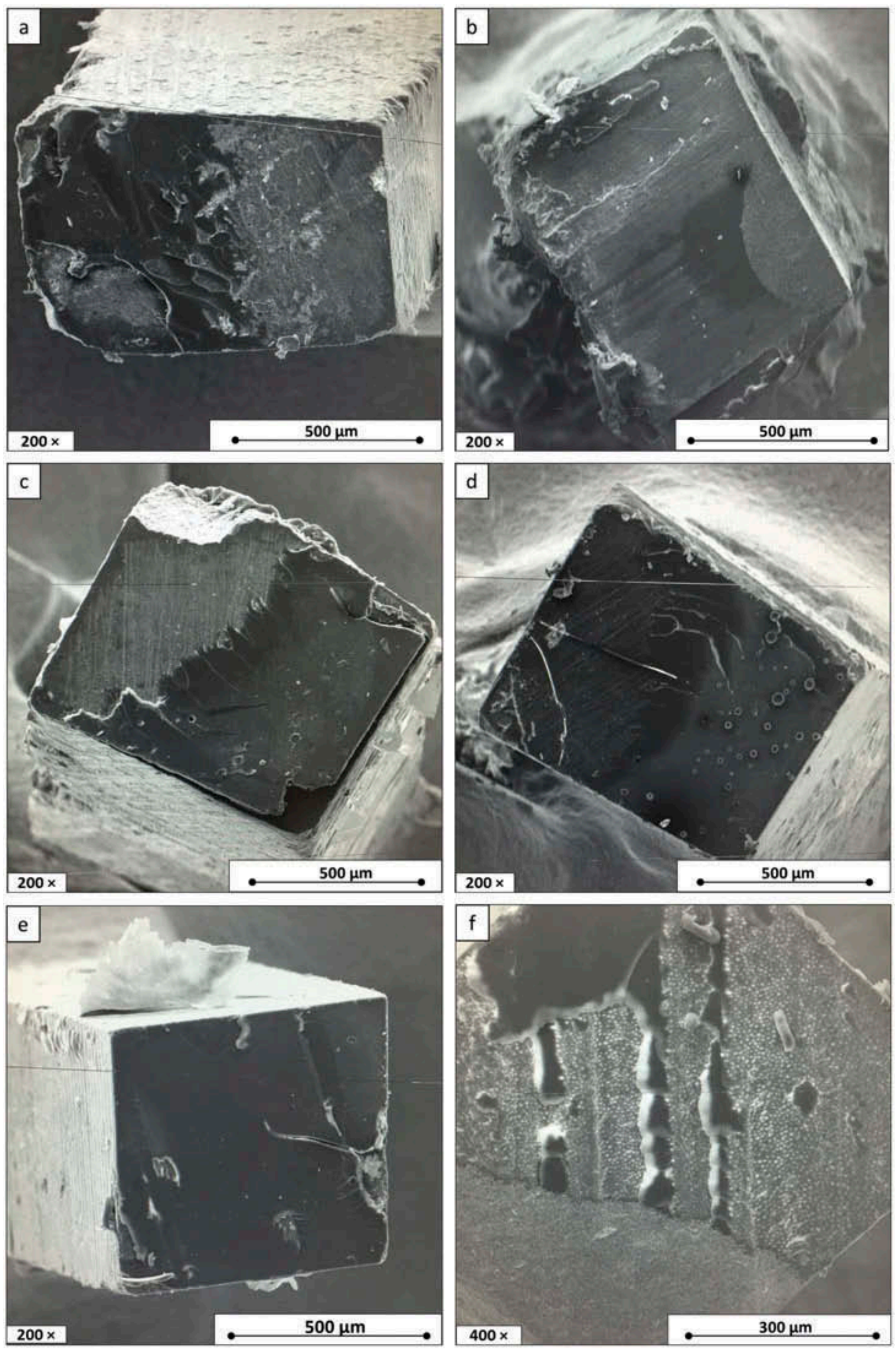

Figure 2. Representatives SEM micrograph of the mode of failure for the fractured specimens after micro-tensile bond strength test. (a, b, c, d) Mixed failure; (e) adhesive failure; (f) High magnification of mixed failure involving composite, adhesive and dentin

is purely water or combined with other volatiles liquids (ethanol, acetone, etc.). In the current study, the utilized adhesive contains water as a solvent. It is known that the water has a low vapour pressure (2.3 kPa at 20°C) that requires prolonged time to evaporate in comparison to any other solvents (Bakhsh, Al-Zayer, Al-Sahwan, et al., 2017; Bakhsh et al., 2013; Qari et al., 2016). Furthermore, according to the manufacturer, the water concentration in Tetric N-Bond self-etch adhesive mixture is about 20–30% of the total volume. It can be speculated that upon adhesive curing, some remnants of solvents might remain and got incorporated within the hybrid layer, while other remnants might exist on the top of the adhesive layer even after polymerization (Breschi et al., 2008, Hashimoto, Tay, Sano, Kaga, & Pashley, 2006). Thus, these solvent remnants would represent potential sites for degradation that would explain the obtained low bond strength for the specimens when they were tested immediately in group 1 (Bakhsh et al., 2013).

Remarkably, there was a significant improvement in bond strength after a 1 min delay. our finding is consistent with a previous report (Asaka et al., 2006) which would be attributed to formation of thick oxygen inhibition layer, which may promote composite adhesion with underlying adhesive as in group 2 (Asaka et al., 2006). It is noteworthy that another *in vitro* study used resin cement on human (Skupien, Porto, Münchow, Cenci, & Pereira-Cenci, 2015) and bovine (Skupien et al., 2015) teeth and did not find the timing has influence on the bond strengths. Based on these results, composite filling application might have different strategy from the resin cement application.

Interestingly, the bond strength of the specimen after 5 min delay had dropped significantly. This could be explained by osmotic water movement over the time through the dentinal tubules into the overlying polymerized one-step self-etch adhesive layer that acts as a permeable membrane (Bakhsh et al., 2017; Qari et al., 2016; Tay et al., 2002). This in turn would affect composite adhesion with the polymerized adhesive layer and explain the relatively high number of premature debonding before testing in this group. It is worth mentioning that SEM images in all groups represented adhesive failures and failures between the adhesive and dentin, and mixed failure (Figure 2). This proves our speculations of the existence of water within the adhesive layer, which might impede a complete adhesion with the overlying composite resin layer.

The current findings would suggest increasing the awareness of dental students and supervising staff in dental schools about the optimum timing for composite application and how it would affect the success of polymeric dental restoration especially in live demonstration educational sessions.

Within the limitation of the study, the null hypothesis was partially accepted as the bond strength in specimens restored immediately or after 5 min were not significantly different, and partially rejected as the bond strength was optimized after 1 min delay and significantly different from the other tested groups.

5. Conclusion

Based on the current study, applying composite restoration after 1 min might improve the bond strength with water-based all-in-one adhesive.

Acknowledgements

This study was supported by Faculty of Dentistry, King Abdulaziz University and Saudi Dental Research group, Jeddah, Saudi Arabia. The authors declare that there are no any known personal, professional, or financial competing interests.

Funding

The authors received no direct funding for this research.

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Citation information
Cite this article as: Time sensitivity associated with the application of water-based all-in-one adhesive system, T.A. Bakhsh, M. Abumansour, M. Shuman, E. Alshouibi & A. Jamleh, *Cogent Engineering* (2018), 5: 1472052.

Clinical relevance
Applying the composite restoration immediately after polymerization of water-based all-in-one adhesive might not be the best timing for improved bond strength.

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