博 士 論 文

The multiple evaluations of the influence of light conditions to "Touch and cure" resin cements - in terms of bond strength test, in situ microhardness test and Raman spectroscopy

"Touch and cure"システム型レジンセメントに対する光照射条件の影響についての多面的検討 - 接着試験, in situ マイクロ硬さ試験およびラマン分光法による重合測定を用いて

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THIS THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE DEPARTMENT OF RESTORATIVE DENTISTRY FACULTY OF DENTAL MEDICINE.

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Abstract

Recently, the new and novel “Touch and cure” technology for dual-cured resin cement was developed and introduced. This new technology is claimed that the polymerization reaction would start immediately after the mixed cement touching dentin surface. This dentin surface is already applied with the primer, containing the new unique added chemical polymerization initiator, before activated by using the light-curing unit.

This study was aimed to evaluate the effect of light and dark conditions to two “Touch and cure” type and one conventional type dual-cured resin cements, in terms of bond strength testing. Nanoindentation microhardness and microRaman spectroscopies were also performed and analyzed to the light condition.

42 flat dentin surfaces polished with #600-SiC were prepared from caries-free human molars and premolars. Then randomly assigned into 3 tests, 1) microtensile bond strength (μTBS), 2) microhardness, and 3) degree of conversion (DC) analysis.

1) For μTBS, 30 molars were randomly divided into three groups: 1. G-CEM ONE (CS), 2. Panavia V5 (PV), both are “Touch and cure” resin cement, 3. RelyX Ultimate (RX); and further divided into two subgroups (n=5) according to light condition for cementation: 1. cement was light-cured from 5 directions for 20 seconds each (L), 2. cement was left in the darkroom for 30 min (D), auto-cured mode. 4-mm resin cement was built-up and cured with each light condition (L or D). Teeth were kept at 37ºC, 24-h storage in distilled water, using a dark box for D-condition. 1 mm^2 non-trimmed beams were tested for μTBS, expressed in MPa. The data were statistically analyzed by Games-Howell test (α=0.05).

2) For microhardness, premolars were cut, smear-layers were prepared and cement built-up and 24 h-water storage same as the μTBS specimen preparation in L-condition previously described. The teeth were cut perpendicular to the resin-dentin interface, 1 mm-thickness slab, and polished with SiC paper and diamond paste down to 1 μm particle size. The microhardness data were evaluated by using the nanoindentation testing machine after specimens polishing 3 and 72 h. The indentation was tested four spots at each 20 μm interval distance from the resin-dentin interfaces until 200 μm. Games-Howell test was used for analyzing the mean values of each group. (α=0.05)

3) For DC analysis by microRaman spectroscopy, the testing slabs were cut and prepared and tested at 3 and 72 h, the same as the microhardness specimens. Each specimen, three lines (1 μm apart) were employed for evaluation Each line started at 2 μm from the resin-dentin interface and tested twenty spots having 1-10μm intervals until 60μm interface distance. One-way ANOVA and Tukey test were used for analyzing means values from each group (α=0.05). Correlation between DC and distance from the resin-dentin interface was analyzed by Spearman’s and Pearson’s correlation.

4) The fracture analysis of μTBS beams after testing was observed and analyzed by SEM. The resin-dentin interface was further investigated by TEM. Filler distribution of cement close to the interface was also analyzed by using SEM.

Results of this study revealed, in L-condition, the μTBS of all cements were not significantly different. Only the μTBS of CS was not affected by the darkroom (D-condition). The μTBS of PV and RX were significantly decreasing in D-condition, approximately 36% and 88%, respectively. There were three pretest failure beams of RX-D. The main failure in this study was mixed failure, except for CS in both conditions which were cohesive failure within cement. In general, we found that adhesive failure was increasing in D-condition.

For hardness and DC analysis results, CSL showed significantly higher values than PVL and RXL. in all testing times. Overall, the means of hardness and DC values of each cement were not significantly different when comparing 3 and 72 h. Except for the hardness of CS at 72 h, was significantly higher than 3 h. Within 2-15 μm distance from the resin-dentin interface, the DC analyses revealed CSL showed a strong positive correlation in both 3 and 72 h, contrast to those of RXL which showed the negative correlation. No correlation was found for PVL.

For correlation of resin filler distribution and the distance from the resin-dentin interface, CSL showed a positive correlation, while RXL showed negative. No correlation was found for PVL.

From the results of this study, it might be concluded that the μTBS of “Touch and cure” type dual-cured resin cement is not affected by the dark condition, while the conventional type does, and showed the higher both microhardness and DC. It might estimate that the “Touch and cure” reaction of mixed cement might probably occur approximately within 15 μm from the resin-dentin interface.

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Background

It has been several decades since the hybrid layer was introduced by Professor Nakabayashi in 1982. [1] Since then, adhesive dentistry developed remarkably for direct restorations, from 3-step etch-and-rinse adhesives to 2-step self etch, and more recently to the multi-mode universal adhesives. Meanwhile, for the indirect restoration approach, the resin cements were also developed.

However, previously before resin cement was introduced, the conventional cement such as zinc phosphate, polycarboxylate and glass ionomer (GI) cement was commonly used for luting the indirect prosthesis. Nevertheless, because of the drawback of this conventional cement type, such as easier solubility when the marginal gap opens between the prepared tooth and the restoration, long working time, or lack of high-level of adhesion. [2–4] As a result, the resin cement was developed and promoted. Resin cements provide many advantages that superior to conventional cement, such as low solubility, optimal bonding to the tooth substrate, superior mechanical properties and so on. [5]

Based on the mode of the initial reaction of polymerization, resin cements can be classified into three types: firstly, light-cured type, activated by the solely light-curing reaction. Secondly, self-cured type, activated by solely chemical reaction, and then lastly, dual-cured type, combined both light and chemical activated reactions. [6]

Light-cured type of resin cements provide the clinical advantages of more desirable demand working, for example, set-on-command capability, and relatively longer setting time by activating cement with the light-curing unit, improved color stability. [7]

On the other hand, self-cured resin cement is another option for these kinds of situations. However, this resin cement type still has the drawbacks, for instance, slow or uncontrollable

working/setting time compared to those of photo-initiator containing in light or dual-cured type resin cement. The cement color tends to change more easily over time, etc. [7]

Dual-cured resin cements also have been widely used and recommended in adhesive dentistry, due to many benefits. Though it shows a lower degree of conversion (DC) as compared to the light-cured type resin cement.[7] However, this type of resin cement was reported about its inferior performance when the amount of light is inadequate. For example, during cementation when the light transmission is difficult or limited to pass through the restoration until reaching the cemented area such as in the case of the metal restoration very high opaque ceramic or in the deep apical part of post space. Even in these cases, dual-cured resin cement is also affected and hampered by an insufficient amount of light, as previous studies have been reported [5,7,8]

For that reason, nowadays, the dual-cured type seems suitable and more convenient to use in many clinical situations. To overcome these drawbacks of the insufficient amount of light issue in this type of cement, companies recently introduced the novel developed with "Touch and cure" technology for dual-cured resin cement. This new modern type is claimed that the polymerization reaction starts not only within the cement content by chemical-initiator when two pastes of cement are mixed together, but also activated by the new additional chemical polymerization initiator in its own cement primer, which prior applied on dentin substrate prior to placing the mixing resin cement. These processes are expected to occur before the photoinitiator is activated by the light-curing unit. This early stage is critical because patient chewing forces can weaken the bonding strength of the prostheses as they are less polymerized, especially in the initial stage after the restoration cementation.

There are reports about the microtensile bond strength (μTBS) of "Touch and cure" resin cement showing higher bond strength and suggesting that it was not affected by light conditions.

[9,10] However, the knowledge about other mechanical properties of “Touch and cure” and its polymerization reaction are still limited.

Nanoindentation or depth-sensing indentation is a powerful technique for measuring the hardness of material at the micro and nanoscales. This device provides both hardness and elastic modulus by measuring from the Berkovich nanoindenter tip. [11]

The DC estimates the quantity of carbon double bonds (C=C) in resin-based material that reacts into carbon single bonds (C-C). DC is determined in relative of the changing before and after material polymerization. DC also plays a key role in both mechanical and chemical properties of resin-based material such as μTBS, microhardness [12], and reaction ability of its chemical composition, resin monomer or the containing initiator.

Thus, the aim of this study is to evaluate how light conditions, especially in the dark condition when the light is limited, affect the bond performance of newly developed type, “Touch and Cure,” compared to conventional dual-cure resin cement by microhardness testing, analyzing the degree of conversion of each material, observing by scanning and transmitted electron microscopies (SEM and TEM).

Chapter 1: Introduction

Resin cement is classified according to the type of chemical reaction, namely, light-activated resin cement, chemical activated resin cement, and dual-cured resin cement. [6,13]

Resin cement is mostly composed with fillers and resin matrix, including resin monomers, silane coupling agents, initiators. [14] The main monomers commonly used in resin cement are high molecular weight resin, bisphenol A-glycidyl methacrylate (Bis-GMA) and urethane dimethacrylate (UDMA), and low molecular weight resin matrix, triethylene glycol dimethacrylate (TEGDMA). Usually, resin cement composes of the mixtures of these different monomers, which link together during the polymerization. [15]

Since light-cured and chemical-cured resin cement adopt different modes initiation for polymerization, by light and chemical reaction, therefore the initiators and activator system in these two cement types also can be different. For the light-cured resin cement, camphorquinone (CQ), which can activate by the visible light, is commonly used as the initiator, together with the organic amine as the accelerator. While the organic peroxide, benzoyl peroxide (BPO), is used as the initiator and using organic amine as the accelerator in the chemical-cure resin cement. While the dual-cure resin cement contains both systems.

Thus, dual-cured system which contains both photo-initiator and chemical-cure initiator has been widely used because this type of cement can use under various clinical situations especially when amount of light during curing process is being questioned or insufficient such as the clinical case with an opaque or thick ceramic or a porcelain fused to metal (PFM) crown or luting the fiber post in the root canal space.

The performance of resin cement has been reported in many mechanical properties aspects, in terms of microtensile bond strength(μTBS), hardness. Many studies show that the light-cured type achieves higher mechanical properties than chemical cured type.[16–22] However, it is not suitable to use the light-cured type resin cement for all clinical cases as the reasons previously mentioned. Additionally, although the dual-cure resin cement, containing both light and chemical cure initiator systems, it also has been reported its properties was impaired from insufficient light because it depends on the photoactivation more than the self-cure initiator. [8,23–25]

Therefore, to overcome this drawback, the novel type of dual-cured resin cement, called "Touch and cure" resin cement, is recently developed and introduced. This type of cement contains not only traditional chemical initiators, such as benzoyl peroxide (BPO), in its paste but also an additional accelerator in its primer which is applied to a tooth surface before cementation. This aimed to help the polymerization start faster immediately after the cement touches with the primer on dentin surface. It is assumed that the fast reaction or polymerization can contribute to a higher DC at the interface and get a higher DC compare to the conventional dual-cured resin cement, which does not contain the accelerator in their primer or adhesive, prior applied to the tooth substrate.

DC is a chemical parameter that plays a key role in both the physical and mechanical properties of resin cement. [12] The polymerization reaction of the resin-based material starts when it is activated by initiator such as CQ or benzoyl peroxide (BPO), and subsequently release the free radicals. The DC is referred to the percentage of unreacted aliphatic carbon double bonds (C=C) converted into carbon single bonds (C-C) to form the polymer network in the cured material concerning the uncured material. The resin-based material should reach a high DC to exhibit better longevity and clinical performance. Various spectroscopies techniques [26–28] have been used to analyze the DC, namely Fourier-transform infrared (FTIR), attenuated total

reflection FTIR (ATR-FTIR), and Raman spectroscopies. The first two techniques are based on absorbing light, while Raman is based on the scattering of light. [29] Generally, DC tends to be evaluated by FTIR or ATR-FTIR methods.

As a result of the difference of spectroscopies techniques, the specimen for FTIR or ATR-FTIR techniques normally requires to be powdered and solely tested the materials or combined with dentin powder [30] or allows to test on the surface but limits to a very thin film resin-based material specimen only. [31,32] On the other hand, Raman analysis, is also reported that it has many advantages compared to the two previous FTIR techniques in terms of easier sample handling and enables multiple measurements due to nondestructive specimens preparation. [28,33,34] Specifically, it can analyze with material only, and also even dentin disc bonded with resin-based material. [27][34]

Moreover, while FTIR can be affected by moisture after the procedure of specimen polishing, the Raman is not. Although, few studies did *in situ* mapping of DC analysis across the resin-dentin interface. [27,35] Most of them tested on the material surface [33], the randomized area in resin cement [6,36] or within the hybrid layer. [37–39]

As a result, these previously reported study protocols may not provide enough data of mixing cement and dentin components in what is relative to the increasing distance from the resin-dentin interface, which is one of our study purposes.

So far, available knowledge on the effects of "Touch and cure" is still limited. [9,10,40] Hence, how the extent of the chemical reaction near the resin-dentin interface occurs and how it changes by time after the polymerization begins are still unclear. Additionally, when dual-cured cements are not properly light-activated, studies showed this situation retards the efficiency of dual-cured resin cement. [8,17]

Therefore the main purposes of this study are 1) to evaluate the effect of two different light conditions, light or dark condition, on three tested dual-cured resin cements in relating to μTBS, 2) to deeply investigate the chemical and physical details of mixed cement in submillimeter-scale close to the resin-dentin interface. The secondary purpose is to introduce the reliable study protocol using the same specimens for *in situ* study on micro indentation hardness and microRaman in relating to micron and submicron increasing resin-dentin interfacial distance.

In summary, this paper reports μTBS of three dual-cured resin cement, two "Touch and cure" type and one conventional type, in two different light testing conditions. For the information of mixed cement near to the resin-dentin interface, we analyzed three different methods and reported: 1) images using scanning and transmitted electron microscopies (SEM and TEM), 2) mechanical property, microhardness, and 3) mechano-chemical properties by microRaman spectroscopic analysis; DC.

The null hypothesis of the study are, 1) the μTBS of two "touch and cure" type and one conventional dual-cure resin cement is not affected by the light conditions and, 2) microhardness and DC of three testing resin cement are not different and affected by time. The alternative hypothesis is that the μTBS, microhardness, and DC of "Touch and cure" type and conventional dual-cure resin cement are different.

Chapter 2: Materials and methods

2.1 Specimen preparation

2.1.1 Teeth

Thirty caries-free human molars and twelve premolars were used in this study. Teeth were stored in 0.5% chloramines-T solution at 4°C and used within 6 months after extraction under the protocol reviewed and approved by the Ethics Committee of Hokkaido University Graduate School of Dental Medicine (#2013-7). Teeth were cut with gypsum model trimmer under water coolant to obtain flat mid-coronal dentin. The light microscope was used to examine no enamel remained on the dentin surface. Then dentin surface was ground with 600-grit SiC paper under continuous water for 60 seconds to create a standardized smear layer prior primer or adhesive using for each cement.

2.1.2 Primer-Adhesive and resin cement

The molars were randomly divided into three groups according to three dual-cured resin cement: G-CEM ONE (CS; GC Corp., Tokyo, Japan), Panavia V5 (PV; Kuraray Noritake Dental Inc., Okayama, Japan), and RelyX Ultimate (RX; 3M ESPE, St. Paul, MN, USA.). Then further divided into two subgroups according to light condition for cementation; under normal ambient light(Light condition; L) and without light curing in a photo darkroom under safe light(Dark condition; D)(n=5). For all groups, primer or an adhesive were applied according to the manufacturers' instruction as shown in Table2 without light cured. The celluloid clear Mylar strip was wrapped around each tooth by using cyanoacrylate glue(Model RepairII Blue, Dentsply-Sankin, Otawara, Japan) to facilitate 4-mm resin cement built up.

For L-condition; cement was light-cured from 5 directions(front, back, right, left and above) at the closest distance from the cement each direction was performed 20 seconds by using a light-curing device(Optulux401, Demetron/Kerr, Orange, CA, USA) at $\geq$ 550 mW/cm^2 and leave under normal ambient light for 10 minutes before stored in distilled water at 37^{o}C for 24 hours.

For D-condition; cement was left in the darkroom for 30 minutes before water storage same as the L-condition subgroup except for the teeth in distilled water were kept in the dark box through 24 hours until taken to cut into the beams.

Table 1 Resin cements used and their application in this study; G-CEM ONE(CS), Panavia V5(PV), RelyX Ultimate (RX)

Group	Dentin Pretreatment	Time (s)	Air blow	Light cure	Resin cement application	Light cured
CSL	Apply tooth primer with agitate motion	10	Strong 5s	No	Hand mixed 4 mm built up	• Optilux 401 (Demetron/Kerr; Orange, CA, USA) at ≥ 500 mW/cm^2 • 5 directions (left, right, front, back and above) 20s each
PVL		20	Mild 10s		Auto mixed 4 mm built up	
RXL						

Table 2 Materials used and their composition formulations

Code	Materials (Lot No.)	Manufacturer	Composition
CSL	G-CEM ONE Adhesive enhancing primer (1607151) 	GC	Water, MDP, 4-MET, MDTP, dimethacrylate, ethanol, initiator
	G-CEM ONE (1607182) 		Fluoroaluminosilicate glass, UDMA, dimethacrylate, initiator, stabilizer, pigment, silicon dioxide, MDP
PVL	Panavia V5 Tooth primer (1L0013) 	Kuraray Noritake Dental	10-MDP, original multifunctional monomer, new polymerization accelerator, HEMA, water, stabilizer
	Panavia V5 paste (6E0033) 		Bis-GMA, TEGDMA, aromatic multifunctional monomer, new chemical polymerization accelerator, dl-camphorquinone, photo polymerization accelerator, others
RXL	Scotchbond Universal adhesive (617265) 	3M ESPE	MDP, Vitrebond™ Copolymer, Bis-GMA, HEMA, ethanol, water, camphorquinone, (dimethylamino)ethyl methacrylate , others
	RelyX Ultimate (625494) 		**Base:** 2-propenoic acid, 2-methyl-, 1,1'-[1-(hydroxymethyl)-1,2-ethanediyl] ester, reaction products with 2-hydroxy-1,3-propanediyl dimethacrylate and phosphorus oxide, TEGDEMA, silane treated silica, others **Catalyst:** Substituted dimethacrylate, 1, 12-dodecane dimethylcrylate, silane treated silica, 1- benzyl-5-phenyl-barbic-acid, calcium salt, others

2.2 Microtensile bond strength (μTBS) test

After 24 h of storage, teeth were longitudinally cut into 1x1 mm beams by a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA) and selected 6 beams at the central area from each tooth (30 beams per group). The beams were fixed to a Ciucchi's jig with the Model repair II. Standard μTBS test was performed in approximate 1 mm^2 non-trim beams verified by a digital caliper (AB204-S Analytical Balance, METTLER TOLEDO, Greifensee, Switzerland) at 1 mm/min crosshead speed by using EZtest machine (Shimadzu, Kyoto, Japan) until the fracture occurred. The μTBS data was expressed in MPa. μTBS and the testing parameter was done at temperature 22–24°C with a maximum humidity of 30%. [41] The beams that failed involving the resin-dentin interface before tensile testing were referred to as pretest failure (PTF) and refer as zero MPa.

2.3 Fracture mode analysis

After μTBS testing, two ends of the fractured specimens were carefully removed from the jig and mounted on an aluminum stub then sputter-coated with Pt-Pd for 120 seconds. The failure pattern of all specimens were determined by scanning electron microscope (SEM; S-4000, Hitachi, Tokyo, Japan). The failure mode on the dentin side of the specimens was classified into as follows, cohesive in cement, cohesive in dentin, adhesive at the interface, and mixed involving interfacial de-bonding. [41]

2.4 Resin dentin interface observation(SEM & TEM analysis)

2.4.1 SEM analysis

The peripheral section slab specimens, perpendicular to the resin-cement interface, from the same teeth of each group during μTBS tested beams produced, were collected. The most center of surface from each group was selected as a representative for resin-dentin interface observation by SEM. The observation surface was subsequencely polished with #600, #800 and #1000 grit silicon carbide (SiC) paper (Sankyo-Rikagaku) respectively under running water then follow by polished with 6 μm, 3 μm and 1 μm diamond paste (DP) (DP-Paste, Struers, Denmark) and cleaned with ultrasonic machine (Fine ultrasonic cleaner, model FU-2H, Gao Hui Mechanical and Electrical International Trade, Nanjing, China) 1 minute for each SiC and DP polishing.

The polished specimens were immersed in 1M hydrochloric acid for 30 seconds rinse with water 1 minute follow by immersed in 5% sodium hypochlorite for 5 minutes and water rinsing for 1 minute. After drying overnight, the specimens were mounted on an aluminum stab, sputter coating, and observed by SEM same procedures as fracture analysis observation as describe before.

2.4.2 TEM analysis

After failure mode was analyzed by SEM, selected beams from each cement in light condition further prepared for non-staining TEM observation. The beams were fixed with 2.5% glutaraldehyde containing 0.1M Sodium cacodylate buffer at pH.7.4 keep overnight at 4ºC. Then rinsed twice with the same solution, each time was 30 minutes. The beams were subsequence dehydrated in each of 70%, 80%, 90%, 95%, and 3 times of 100% ethanol and embedded in epoxy resin (Epon 812, Polysciences, Inc., Warrington, PA, USA). The 75-90 nm thickness of the specimens were obtained by using a diamond knife (Diatome, Bienne, Switzerland) in an ultramicrotome (Ultracut, UCT, Leica, Vienna, Austria) cut through the resin-dentin interface. The sections were observed with a transmission electron microscope (TEM; H-800, Hitachi, Tokyo, Japan) operating at 75 kV. [42]

2.5 Micro indentation hardness analysis

We used the nanoindentation test to evaluate the hardness data in this study because the force was loaded in the mN range. The indentation mark achieves from this method could be small size down to micron and in the order of nm in depth [43,44] consequently, enable to analyze at the tiny area closed to the resin-interface.

The additional three flat dentin surfaces polished with #600-SiC paper were obtained from caries-free human premolars. Each tooth was randomly assigned to three groups(n=1) and prepared according to the conventional μTBS specimen preparation as prior described before. After light curing, the specimens were left for 10 minutes before water storage at 37ºC in distilled water for 24 h.

For making approximately 1.2 mm testing specimens, teeth were sectioned at the middle in a mesiodistal direction along the long axis of the teeth and perpendicular to the resin-dentin

surface. The testing specimens' surfaces were subsequentially polished with SiC paper and diamond paste (DP-Paste, Struers, Ballerup, Denmark) down to 1-µm particle size. The polishing step was carefully done for producing the well uniform smooth surface specimens. Then they were dried 3 h before hardness tested. Micro indentation hardness test was performed at 3 h and 72 h after polishing.

For the selected 3 and 72 h testing time, the pilot studies were done before setting the testing protocol. The data are not shown here. Attributable to Almas et al. in 2019 [45], they demonstrated that the wetness of both dentin and resin-base material would be able to affect the physical properties, hardness value, of specimens by time. Therefore, to minimize this concern, pilot studies were done before establishing the microhardness analysis procedure. After complete polishing to 1-µm particle size, the well-polished resin-dentin specimen were observed their weight by using a digital balance (METTLER TOLEDO, AB204-S Analytical Balance) every hour until no more weight loss from water during polishing procedure. In our pilot studies, it takes approximately at least 3 h for the dual-cured resin cement-dentin slab specimens to have a stable of their weight. Furthermore, in another pilot study, we found the specimens' hardness value of 3 days (72 h) and 7 days were not different. Thus, we selected 72 h being as one of the testing periods as the polymerization of dual-cured resin cement is ongoing over time.

The microhardness was measured with an ultramicro hardness tester (DUH-211; Shimadzu), under the average load of 5 mN at a speed of 0.2926 mN/s; the holding time at peak load was 10 seconds. The indenter, Berkovich type diamond tip, is a triangular pyramidal diamond shape with a tip angle of 115° and radius 0.1 µm.

Microindentation Martens hardness (HM) was calculated by dividing the maximum force by the projected area and then converted to MPa. The hardness values were obtained from the

default software of the testing device with using the Oliver and Pharr method [44] as the following;

$H = \frac{P\ max}{A}$ when H: hardness, *P* max: maximum load and A: contact area

The tests were performed starting from dentin then passing across the interface toward the cement. The vertical distance between each tested area is 5µm in dentin and 10µm in cement. The hardness values of resin cement were averaged at each 20-µm interval distance from the resin-dentin interface until 200-µm depth. A minimum of 10 µm horizontal distance was maintained between adjacent indentation. Data achieved 4 indentations per each 20 µm interval. In total, 40 indentations (n=40) were performed on the specimens per testing time (Figure1). The dentin was tested to establishing the line for indentation. Therefore, their data were not analyzed. Samples were tested in the range of ambient temperatures 22–24°C with a maximum humidity of 30%. After tested at 72 h, the hardness tested specimens and additional resin-dentin specimens from the same tooth were further sputter-coated and observed under SEM same manner as for resin-dentin interface observation.

Game-Howell test was used to statistically analyze the hardness values as the variable among different cement (CSL, PVL, and RXL) and two testing time interval, 3 and 72 h, at 0.05 level of confident ($\alpha = 0.05$).

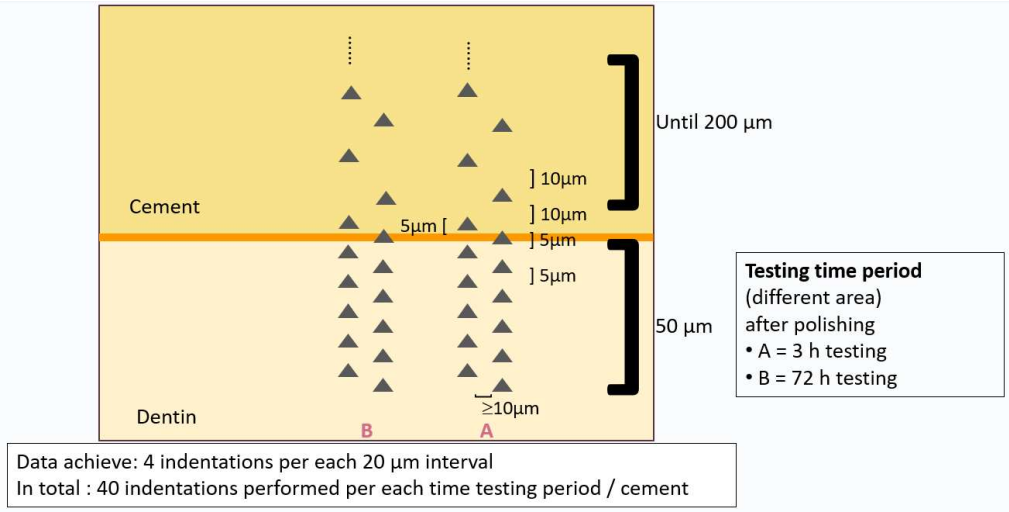


Figure 1 The diagram shows testing micro indentation hardness protocol; the triangle shapes refer to the indenter marks. Two vertical patterns, A and B, are the employment of the indenters in each testing interval; A: 3h, B: 72h.

2.6 MicroRaman and Degree of conversion (DC) analysis

For micro-Raman analyses, additional three teeth per cement (CSL, PVL, and RXL) in light condition; n = 3, in total nine premolars, were cement-built up, cut and polished as micro indentation specimens preparation as previously described. The specimens were dried at room temperature for 3 h before micro-Raman analyses and subsequently kept in dry and dark condition until tested again at 72 h, same testing time interval as the microhardness test in section 2.5.

Both the polymerized and non-polymerized resin spectra were acquired by using a MicroRaman spectrometer (Renishaw inVia Reflex Raman Microscope; Renishaw PLC, UK) with the following setting: 785 nm semiconductor laser, laser beam size of 1 µm, 30 mV, 1200 grooves/mm diffraction grating and a 50X objective. All spectra were ranged between 700 and 1850 cm^{-1}, with a spectral resolution of 1 cm^{-1}, an exposure time of 1 s, and 5 accumulations.

Each specimen was measured across the section in 28 depths from the interface (reference: 0 µm) to the dentin at -10, -5, -4, -3, -2, -1 µm distances and continuing measured in cement at

intervals of 1 µm steps until 15 µm (+1 µm) and subsequently at +20, +25, +30, +40, +50, and +60 µm. In each specimen, three lines (100 µm apart) were employed for evaluation, with the central line normal to the highest pulp horn (Figure 2). The three lines data per each depth were used for calculating the average degree of conversion (DC).

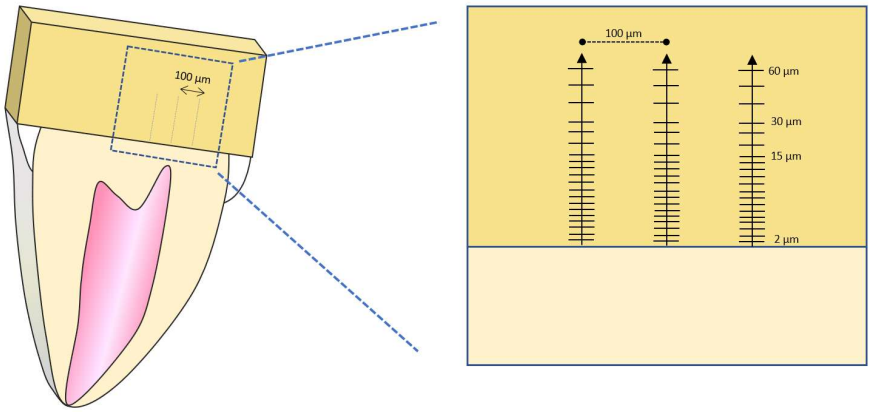


Figure 2 Resin-dentin specimens and protocol for MicroRaman analyses. Three lines data were measured from the interface (28 depth steps) for the evaluation of DC values.

The DC values were evaluated by determining the polymerized specimens in the term of changing of peak height ratio of the absorbance aliphatic C=C at 1639 cm^{-1} and the internal reference peak of aromatic C=C at 1609 cm^{-1} which will not change during polymerization, concerning the unpolymerized material. Except for CSL material, the peak of aliphatic C-H at 1455 cm^{-1} was used as the internal reference peak. The DC values for each specimen were calculated according to the following formula: $DC\% = \{1- \frac{\text{R cured}}{\text{R uncured}}\} \times 100\%$

$$DC\% = \{1- \frac{1639\ cm^{-1} / 1609\ cm^{-1}\ cured}{1639\ cm^{-1} / 1609\ cm^{-1}\ uncured}\} \times 100\ \%\ \text{ for PVL and RXL}$$

$$DC\% = \{1- \frac{1639\ cm^{-1} / 1455\ cm^{-1}\ cured}{1639\ cm^{-1} / 1455\ cm^{-1}\ uncured}\} \times 100\ \%\ \text{ for CSL}$$

2.7 Percentage (%) ratio of primer or adhesive to cement analysis

Additional resin cements materials were tested each component of primer or adhesive and cement separately, for example, cement paste A, paste B, mixed cement, the primer or adhesive. A pair of relative Raman spectrum band was used for analyzing the variation of primer or adhesive amounts to the dentin prior bonded with resin cement. These spectrum bands were selected correspond to each of the peaks in primer or adhesive (yellow line) and mixed cement (black line) component (Figure 3). Each band for primer and adhesive or cement was carefully selected based on the peak that shows the high intensity one component, while as shows less intensity in the other component, primer or cement. Relative peak intensities used were 880/750 (CSL), 905/1113(PVL), and 883/1003 (RXL), the blue letter labeled in each cement graph, as shown in Figure 3 and Table 3.

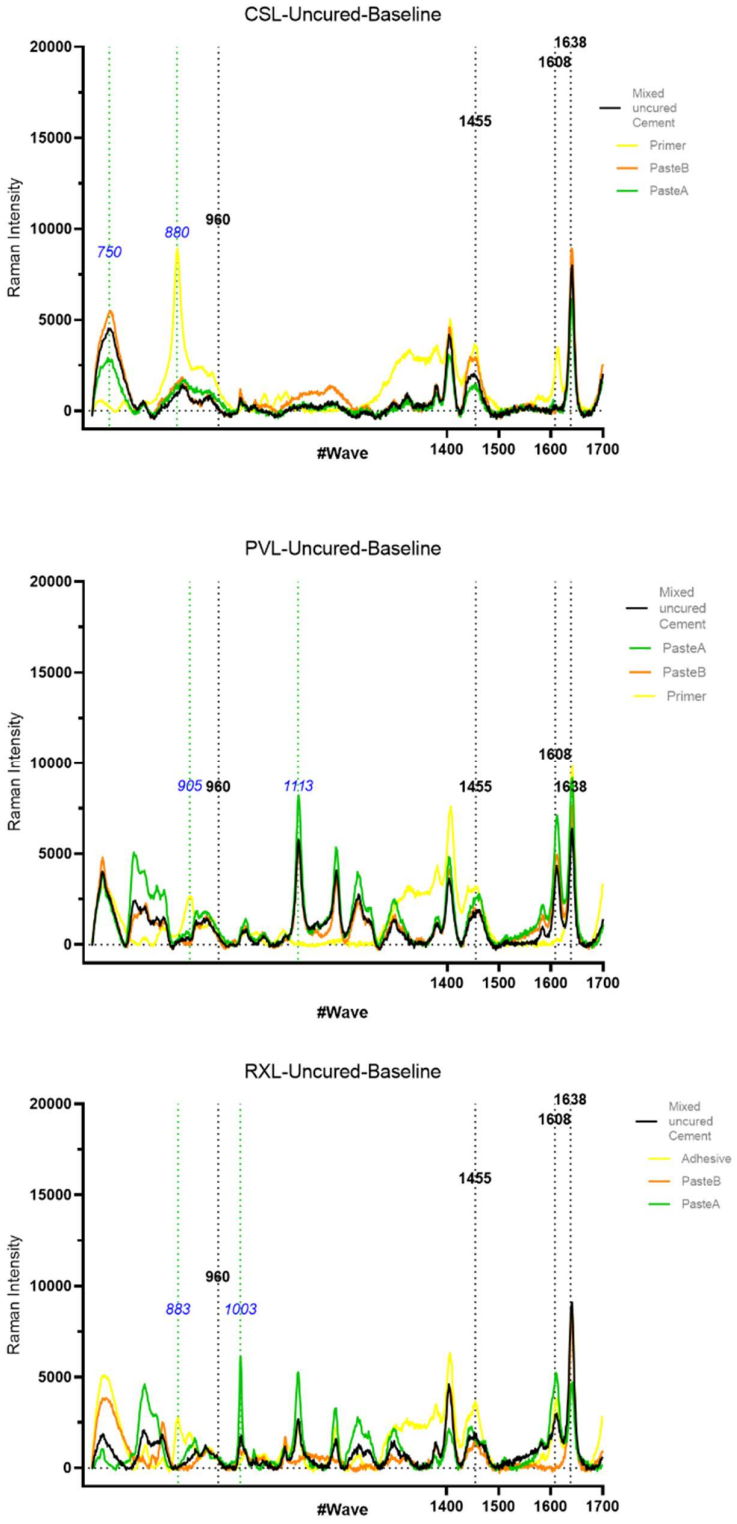


Figure 3 Relative peak intensities of uncured cement; black, primer; yellow, paste A; green and paste B; orange, of each resin cement. CSL; G-CEM ONE, PVL; Panavia V5, and RXL; RelyX Ultimate.

Table 3 The following ratios represent the degree of conversion of material per each cement

Cement	Changing peak band	Internal reference peak band		Relative band used	
	Aliphatic C=C	Aromatic C=C	Aliphatic C-H	% DC	Primer/cement ratio
CSL	1639		1455	1639/1455	880/750
PVL	1639	1609		1639/1609	905/1113
RXL	1639	1609		1639/1609	883/1003

Each spectrum was automatically removed from its background and corrected baseline by using Renishaw's WiRE software (Renishaw PLC, UK) before comparing of remaining C=C double bond in the formula mentioned above.

2.8 Percentage (%) ratio of resin matrix analysis

One of the SEM images(x1500) from each cement up to 20 μm from the resin-dentin interface was analyzed using Image J software version 1.52. These images were converted to grayscale in 8-bit and carefully adjusted the threshold for measuring pixels and calculating as % resin matrix. During threshold adjusting, the dark area, meaning low-value pixels that represent resin matrix, will turn red, but those represent filler will not change. The threshold values used for analyzing were approximately 26 %, 40 %, and 46 % for CSL, PVL, and RXL, respectively.

We used this resin matrix-fillers analysis method by modified the measured area using the thresholding method recommended by the Science Education Resource Center (SERC) at Carleton College for measuring distance and area in the satellite in "A Case study of The Shrinking Aral Sea." Briefly, first, they make measurements from digital satellite images, set the scale calibration of an image. In our case, we use the scale in a picture that automatically generated from the SEM machine. Then select the distance to measure, in our case we set a square area (2 x 2 μm) as a single measurement and measured 10 times in the height of 20

μm and 5 times in width of 10 μm, in total the area we analyzed each specimen is 200 μm^2 (h20 x w10 μm^2). Then adjust the threshold and convert the dark pixels, representing water in the satellite map or as resin matrix in resin cement, will have low values and the lighter pixels, representing land or resin filler in resin cement, will have higher values. Then the low-value pixels that represent water, or resin matrix, turn red, but those that represent land don't change. Next, the red area will automatically calculate. For each % resin matrix value until 20 μm from the resin-dentin interface, we use the average of 5 squares data in each 2-μm interval height.

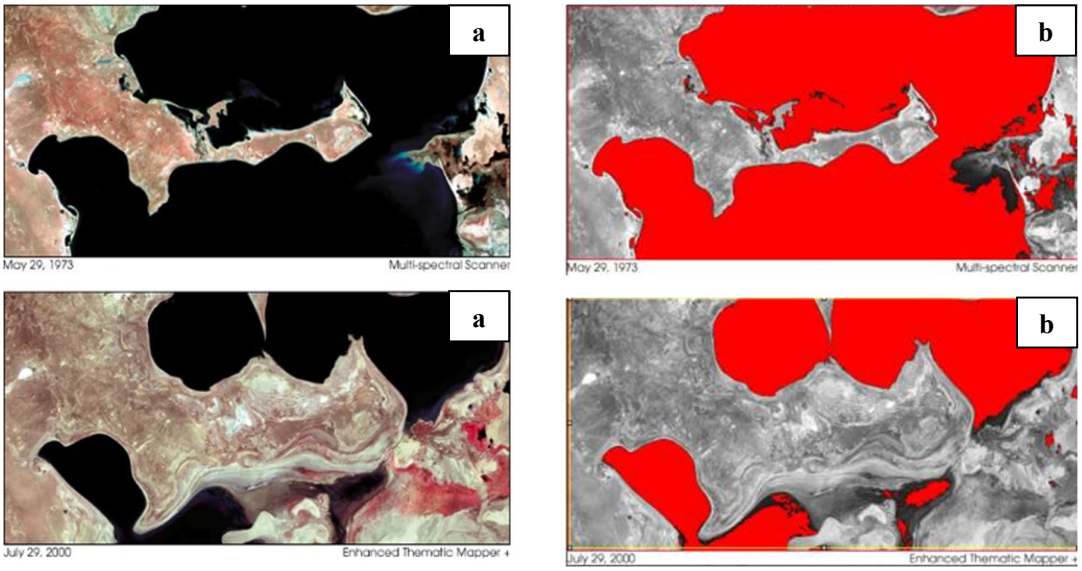


Figure 4 The measurement area in digital images by using threshold adopt and modified from the Science Education Resource Center (SERC) at Carleton College. [46]

(a) The sequence of images shows the dramatic changes to the Aral Sea between 1973 and 2000. (b) The Aral sea has adjusted the threshold and converted. The dark pixels in Figure 3a are low-value pixels that represent water turn red, but those that represent land don't change. The red area will automatically calculate.

2.9 Statistical analysis

For each test (μTBS, Microhardness, and %DC), data were analyzed with Shapiro-Wilk and Levene's tests to check normality and Homogeneity of variance, respectively.

For μTBS and microhardness, the data were analyzed by non-parametric Games-Howell.

For %DC analysis, the means of DC of each cement at 3 and 72h were analyzed by One-way ANOVA and Tukey test ($\alpha = 0.05$). Pearson's correlation test ($p < 0.05$) was done to analyze the relation between DC and distance from the resin-dentine interface for CSL and RXL, while PVL was analyzed with Spearman's correlation test ($p < 0.05$) as distribution was not normal.

All tests were performed with SPSS version 22.0 for Windows (SPSS, Chicago, IL USA).

Chapter 3: Results

Microtensile bond strength (μTBS) test and fracture mode analysis

The result of μTBS and failure analysis are shown in Figure 5 and 6. CS-L revealed the highest bond strength (61.6 ± 18.69 MPa), while RX-D is the lowest (6.7 ± 5.71 MPa). For the L-condition, μTBS was not affected by the type of cement. For the D-condition, CS showed the highest bond strength values among all cements, followed by PV and RX. There were 3 pre-test failure beams in RX-D; the data refer to zero MPa. All groups showed significant differences when L- and D-condition were compared, except for CS.

When primer or adhesive and cement were applied in the darkroom under safelight, dark condition, adhesive failure was increased in all cement except for RX. Figure 7 shows representative SEM images of predominant failure in each group from Figure 6.

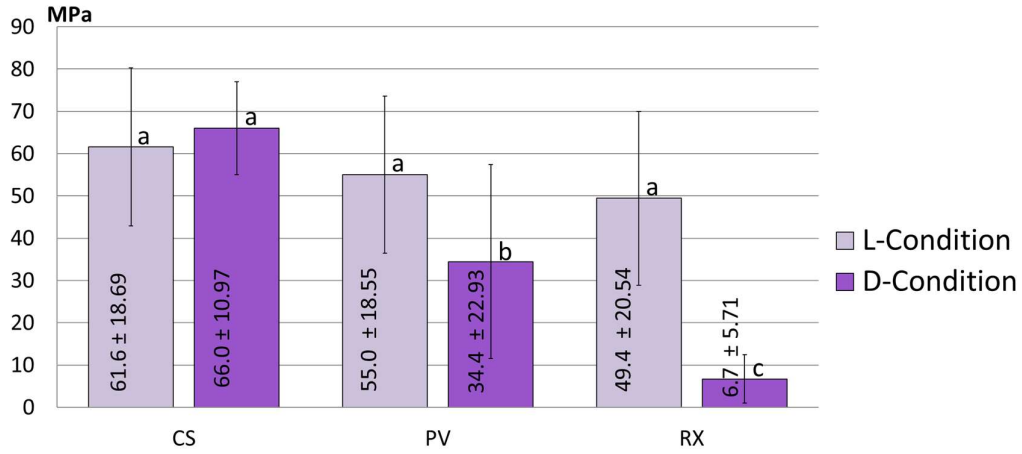


Figure 5 Means microtensile bond strength (μTBS) ± standard deviations (SD) of the experimental groups. Same letters are not statistically different ($p > 0.05$)

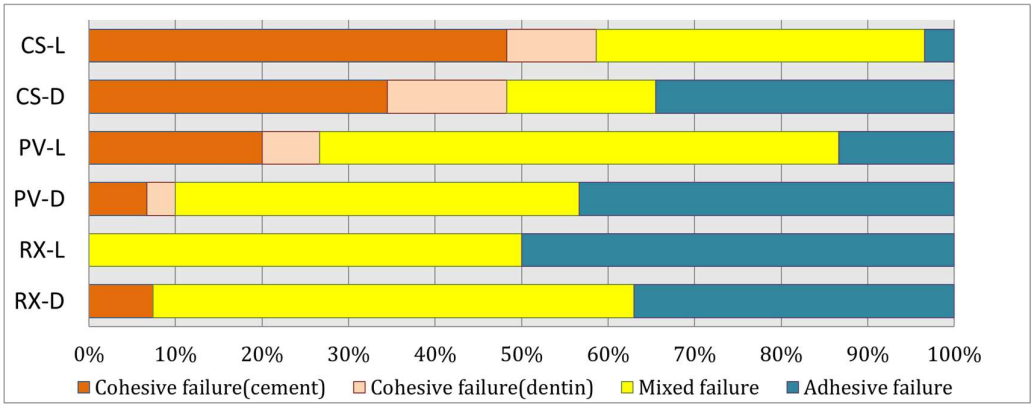


Figure 6 Graph presenting failure mode distribution

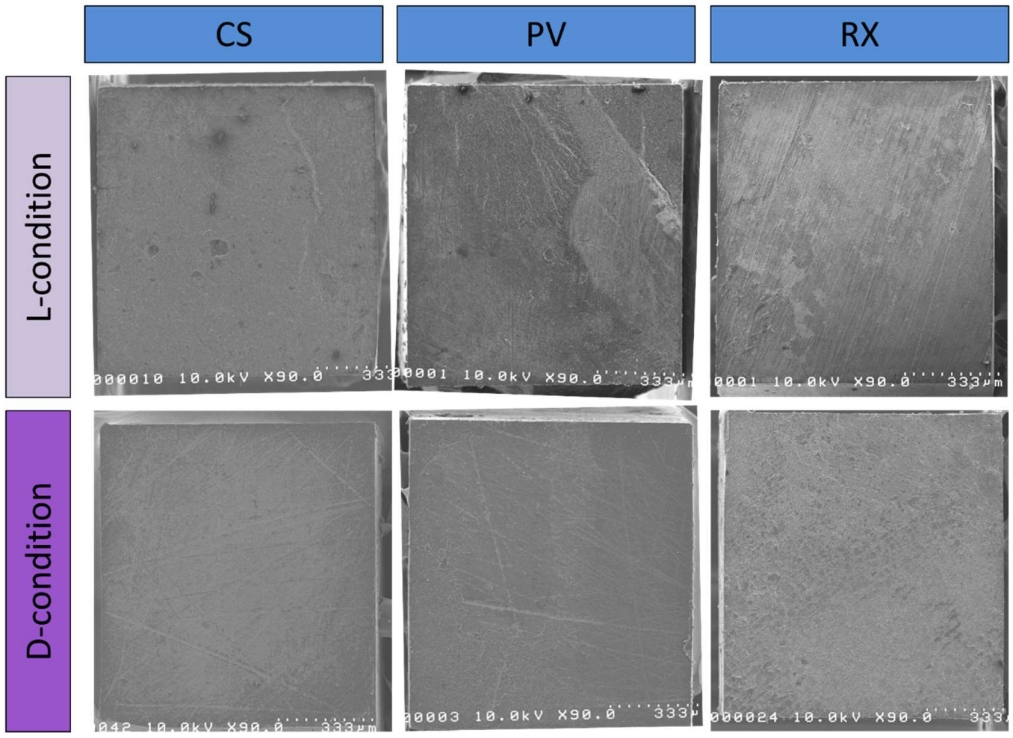


Figure 7 SEM images of the fractured beams after testing (dentin side), representing the predominant failure of each cement. Cohesive failure in cement; CS-L. Adhesive failure; RX-L and CS-D. Mixed failure; PV-L, PV-D, and RX-D.

Resin dentin interface observation(SEM & TEM analysis)

SEM analysis

Based on SEM images in Figure 8, resin tags in CS are uniform distribution and more than those in PV and RX group. Moreover, resin tags were reduced by dark conditions for PV and RX.

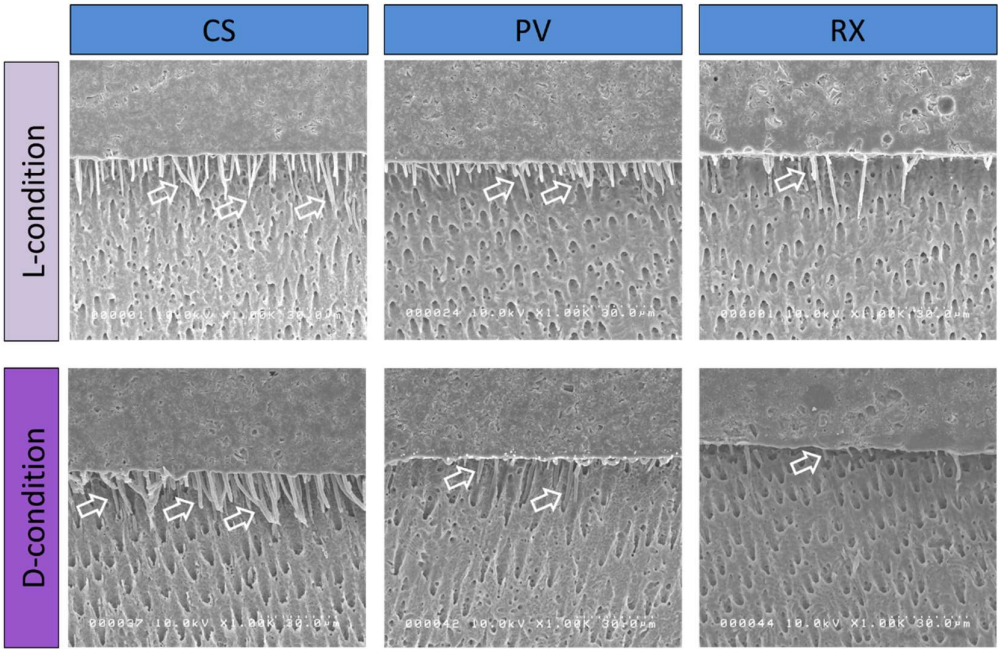


Figure 8 SEM images of the resin-dentin interface. Resin tags in CS are uniform distribution and more than those in PV and RX group. The arrows show the resin tags.

TEM analysis

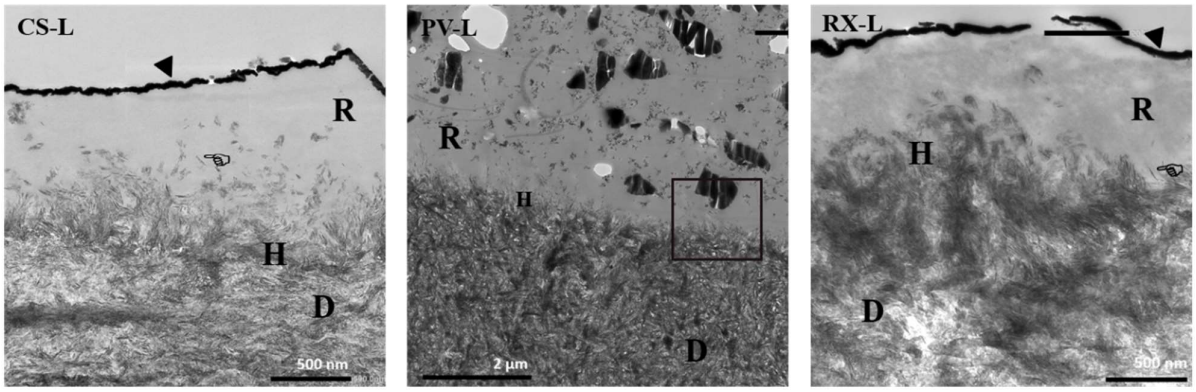


Figure 9 TEM images of the resin-dentin interface of fracture specimen of G CEM ONE(CS-L), Panavia V5(PV-L), RelyX Ultimate (RX-L). The black triangles in CS-L and RX-L showed the spatter coating materials used when previously observed these fractured specimens with SEM.

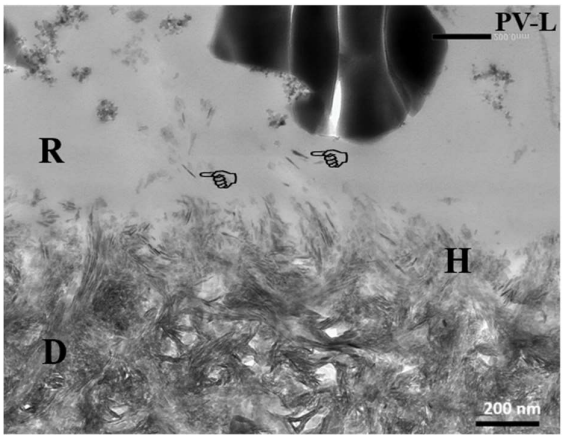


Figure 10 A higher magnification (x50,000) TEM image of the area within the box Figure 9 of PV-L. Hand pointers show hydroxyapatite crystal.

Micro indentation hardness analysis

This bar chart shows the result of the hardness of all testing cements at 3 and 72h. CSL at 72 h showed the highest hardness value among all groups. Regardless of the curing mode, only the hardness values of CSL showed a significant difference when periods were compared.

Among touch and cure resin cements, CSL showed higher hardness value than PVL in both 3 and 72 h testing time. (Figure 11). The graphs in Figure 12 shows the average of the hardness values of three cement, by each depth starting from the resin-dentin interface at 3 and 72 h. Figure 13 demonstrates the SEM image of the resin-dentin specimen after micro indentation hardness testing. The yellow stars represent dentinal tubules, PD is peritubular dentin. The size of indentation marks is approximately 5 µm, indicated with hand pointer.

Figure 11 This bar chart shows the result of the hardness of 3 cements at 3 and 72h. CSL at 72 h. The connected bars indicated no significant difference.

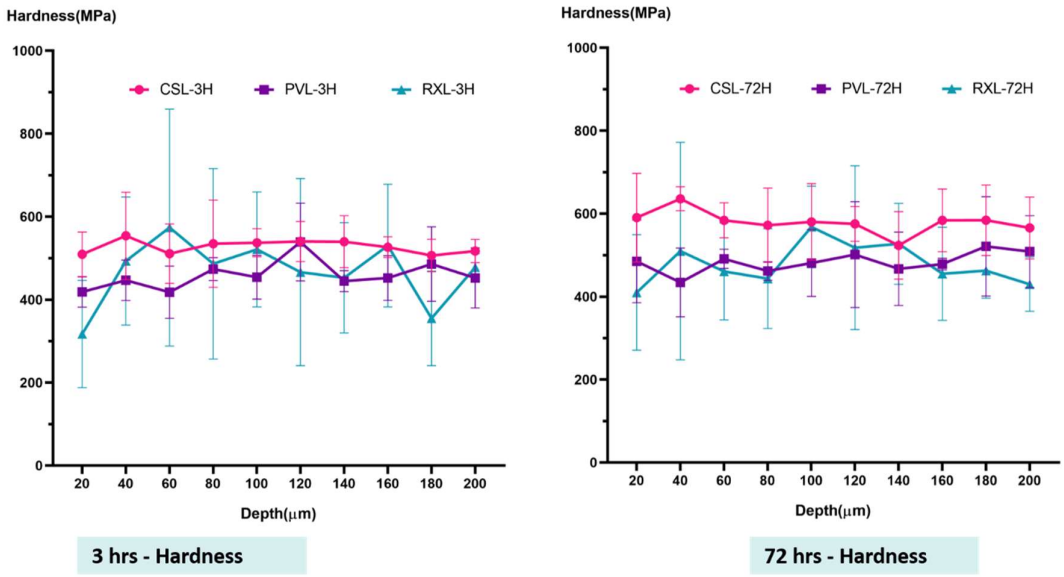


Figure 12 The graphs show the average of the hardness values of three cement; CSL, PVL, and RXL, by each depth starting from the resin-dentin interface at 3 and 72 h.

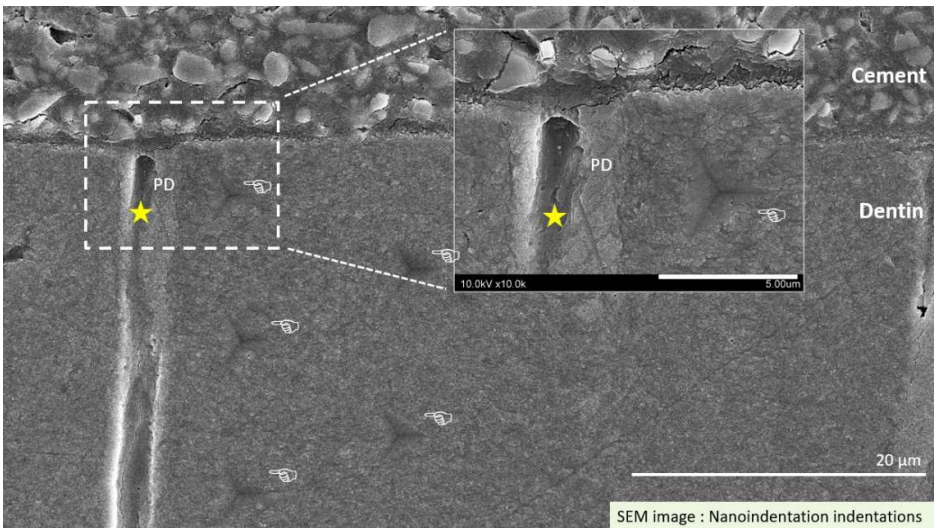


Figure 13 SEM image of a specimen after testing micro indentation hardness.
The yellow stars indicate the dentinal tubule. PD is peritubular dentin. The hand pointers show the indentations according to the testing pattern. The size of the indentations is approximately 5 µm.

MicroRaman and Degree of conversion (DC) analysis

Within 60 µm from the resin-dentin interface, CSL demonstrated the highest mean DC, which was statistically different from RXL and PVL in both testing time ($p < 0.05$), showing PVL the lowest DC values (Figure 14). No differences over time (3 h and 72 h) were showed for DC values of all cements. Mean DC ± standard deviations (SD) in % of CSL at 3 and 72 h are 88.56 ± 3.9 and 88.61 ± 4.2, respectively. For PVL at 3 and 72 h are 77.69 ± 0.4 and 77.71 ± 0.7, respectively. For RXL at 3 and 72 h are 80.42 ± 1.7 and 81.24 ± 0.7, respectively.

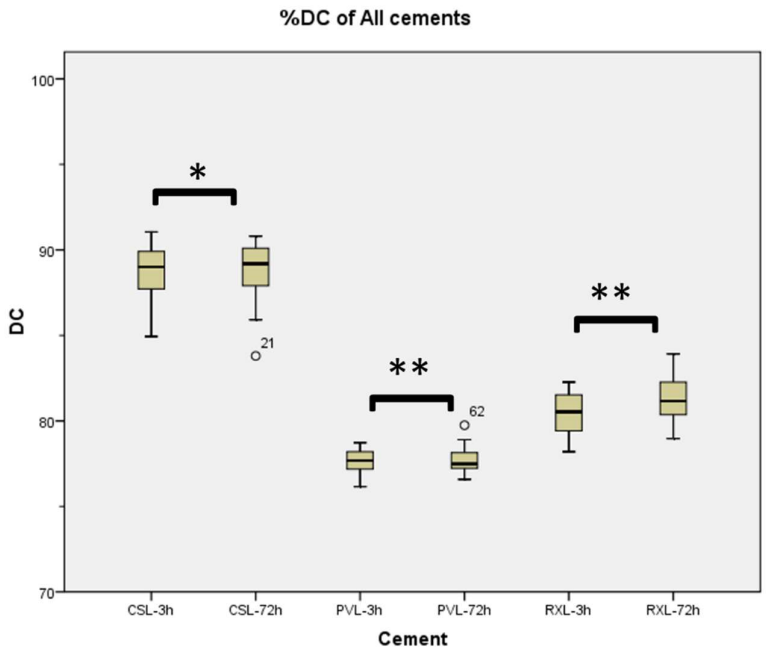


Figure 14 The boxplot shows the mean %DC of each cement at 3 and 72h. **Same symbols are not statistically different ($p > 0.05$)**

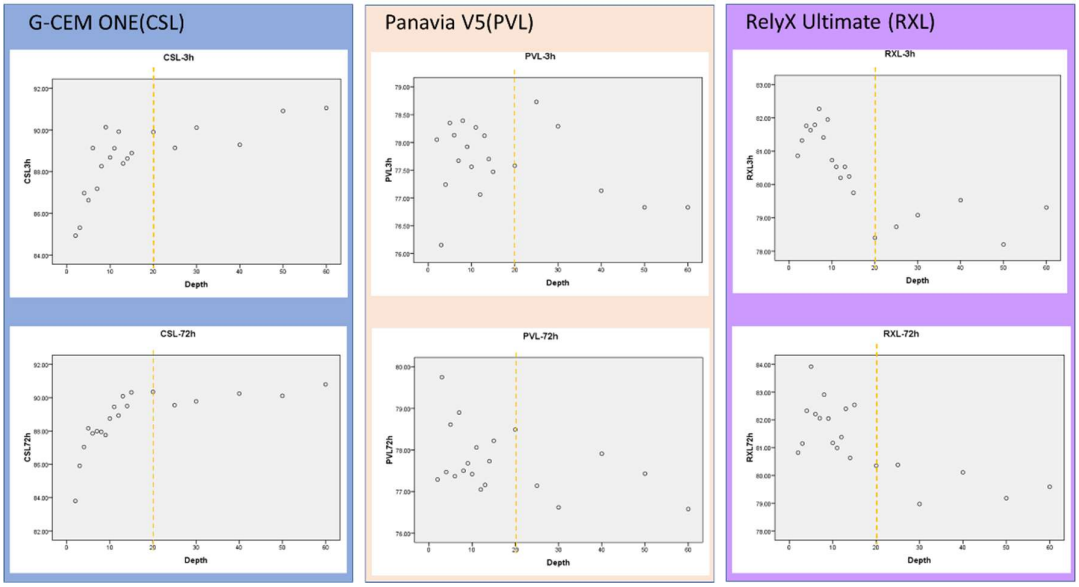


Figure 15 Correlation of %DC and resin-dentin interfacial distance (2-60 µm) of each cement at 3 and 72 h. The yellow lines demonstrate at depth 20 µm from the interface.

Figure 15 reveals the strong linear relationship was determined between DC and the 2 - 15 µm distance from resin-dentin interface for CSL, Pearson's correlation (r = 0.75; $p = 0.002$) and (r = 0.89; $p < 0.001$) for 3 and 72 h respectively. Negative correlation was found in RXL at 3h, Pearson's correlation (r= -0.69; $p = 0.006$), while there was no correlation at 72 h.

For PVL, both 3 and 72h, there was no linear relationship between DC and the distance, Spearman's correlation ($p > 0.05$). (Figure 16)

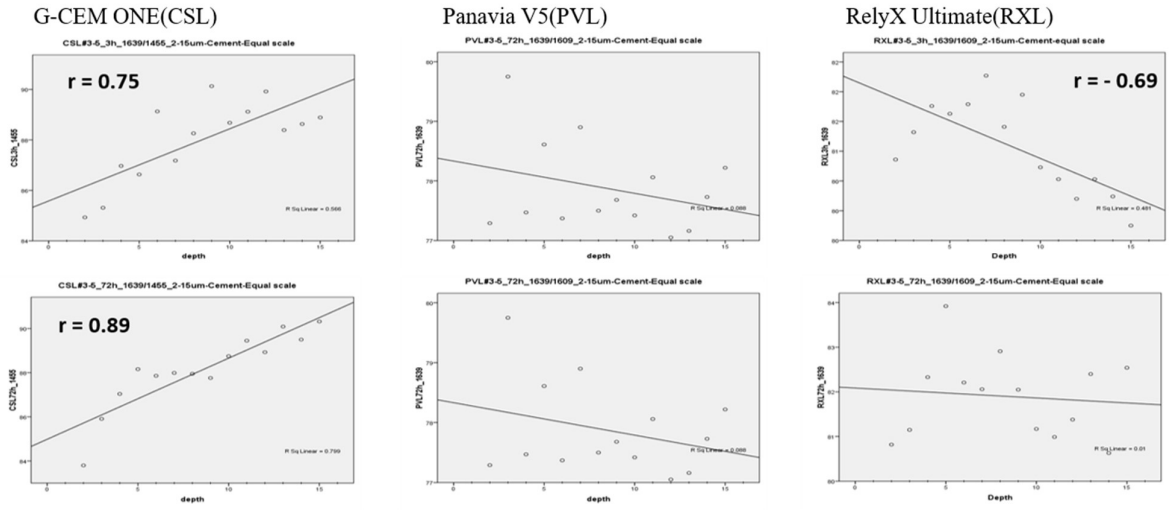


Figure 16 Correlation of %DC and resin-dentin interfacial distance (2-15 µm) of each cement at 3 and 72 hours.

% ratio of primer or adhesive/cement analysis

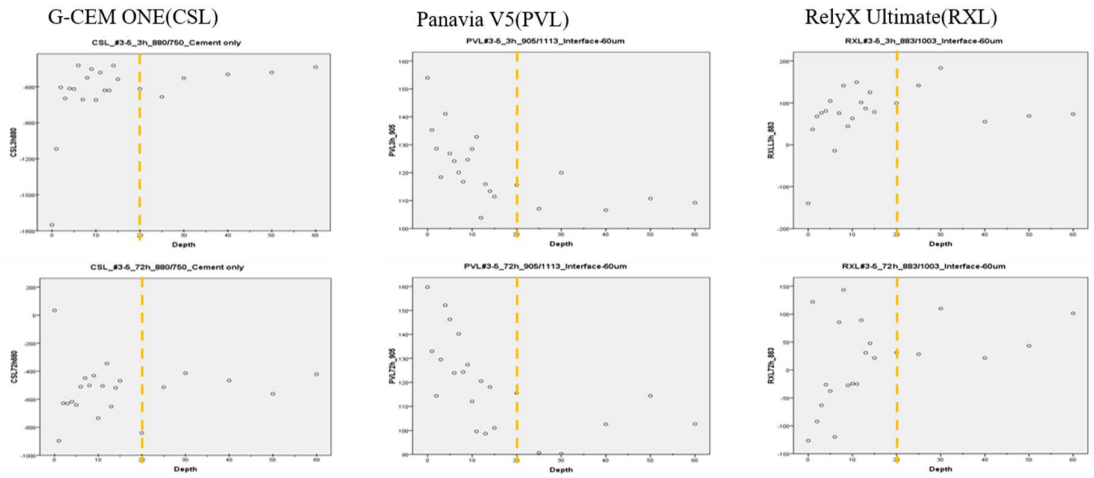


Figure 17 Correlation of primer/cement ratio and resin-dentin interfacial distance (2-60 µm) of each cement at 3 and 72 h. The yellow lines demonstrate at depth 20 µm from the interface.

When analyzing the relationship between the changing of primer/cement ratio and the distance from the resin-dentin interface from 2 – 15 µm (Figure 17 and 18), only Panavia V5 group were found a negative correlation in both 3 and 72 h. Pearson's correlation showed at 3h (r = - 0.60; p = 0.022) and at 72 h (r = -0.64; p = 0.013). However, there was no linear relationship for G CEM ONE and Rely X Ultimate in both 3 and 72 h.

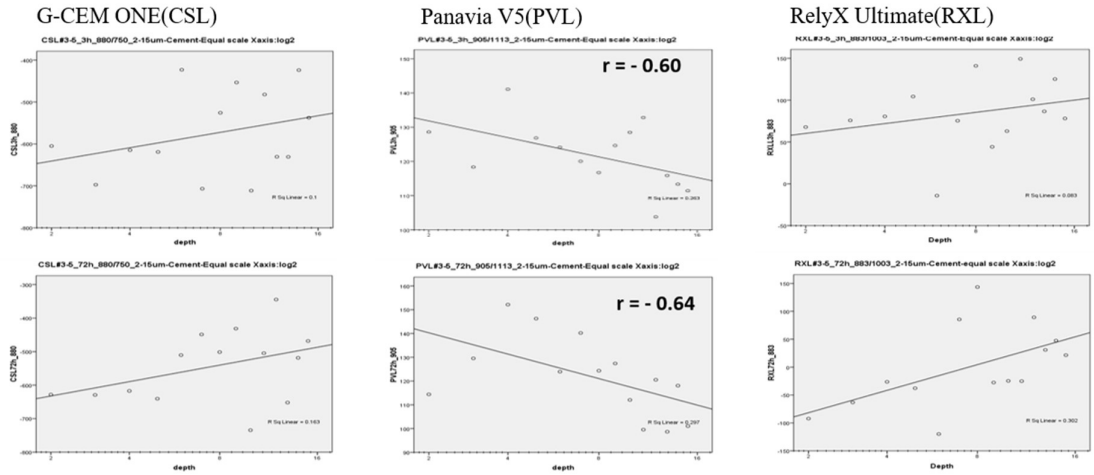
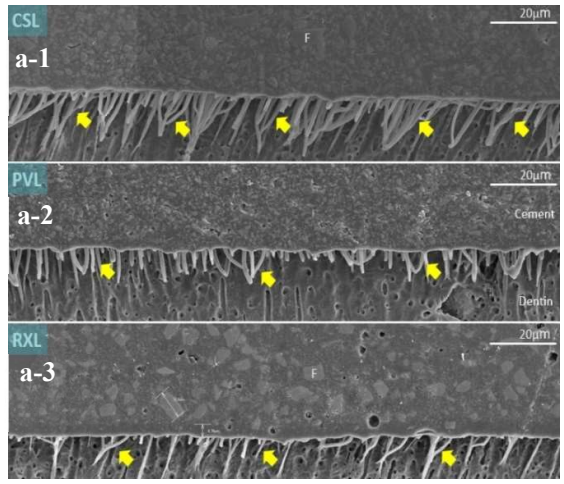


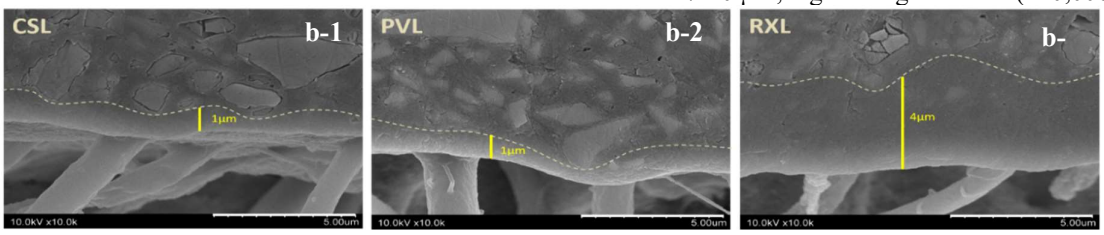
Figure 18 Correlation of primer/cement ratio and resin-dentin interfacial distance (2-15µm) of each cement at 3 and 72 h.

SEM image of a specimen after testing microRaman

Based on these pictures, resin tags in CSL are more in number and uniformly distributed than those of PVL and RXL groups. The F letter shows the fillers. RXL has the biggest filler, whereas PVL has the smallest. CSL's filler distribution is more homogenous than those of RXL (Figure 19a). These big fillers are uniformly distributed, and their size is nearly 7-10 µm, as shown in Figure19c.

From the higher magnification (x10,000) of the same specimen in the Figure 19a, they showed the thin layer of primer, G CEM ONE, and Panavia V5, approximate 1µm. While an adhesive layer of RXL, using RelyX Universal adhesive, is thicker, approximately 4-5 µm (Figure 19b).





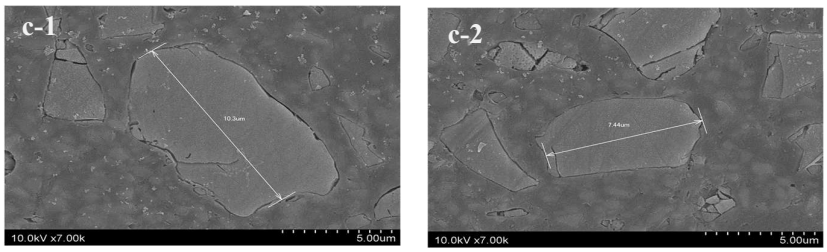


Figure 19 (a1-3) SEM images (x1500) of each resin cement, the same specimen after testing micro Raman. Arrow; Resin tag, F; Filler. For RXL, some fillers are fillers that are the biggest among all cement. These big fillers are uniformly distributed, and their size is nearly 7-10 µm, as shown in (c).

(b1-3) Higher magnification (x10,000) of the same specimen in (a) shown the thin layer of primer, G CEM ONE, and Panavia V5, approximate 1µm. While an adhesive layer of RXL, as using RelyX Universal, is thicker, approximately 4-5 µm.

(c1-2) Some of resin fillers of RXL in Figure a-3 are sized 7-10 µm, higher magnification (x10,000).

% Resin matrix analysis

The average percentages of resin matrix ratio to resin filler from the resin-dentin interface until 20 µm. SEM (x1500) of each cement by using Image J software is displayed in Figure 20 a-c. From these SEM pictures, each cement shows the different characteristics of resin matrix distribution.

CSL show resin matrix distribution is decreasing, on the other hand, resin filler is increasing when the far from the resin-dentin interface. Consequently, CSL seems to have a positive tendency of filler distribution to the distance from the resin-dentin interface.

In contrast, RXL, the resin matrix is increasing, on the other hand, resin filler distribution decreases. The filler distribution of this cement seems to have a negative tendency to the interfacial distance. Except for at 2 and 4 microns (Figure 20 c-3), the bars show the high % of the resin matrix, 82.12 and 54.98%, respectively. We assume they are the adhesive layer in the mixed cement, similar thickness (Figure 19 b-3 and 20 a-3).

For PVL, the bar chart shows the resin matrix is uniform distribution (Figure 20 c-2).

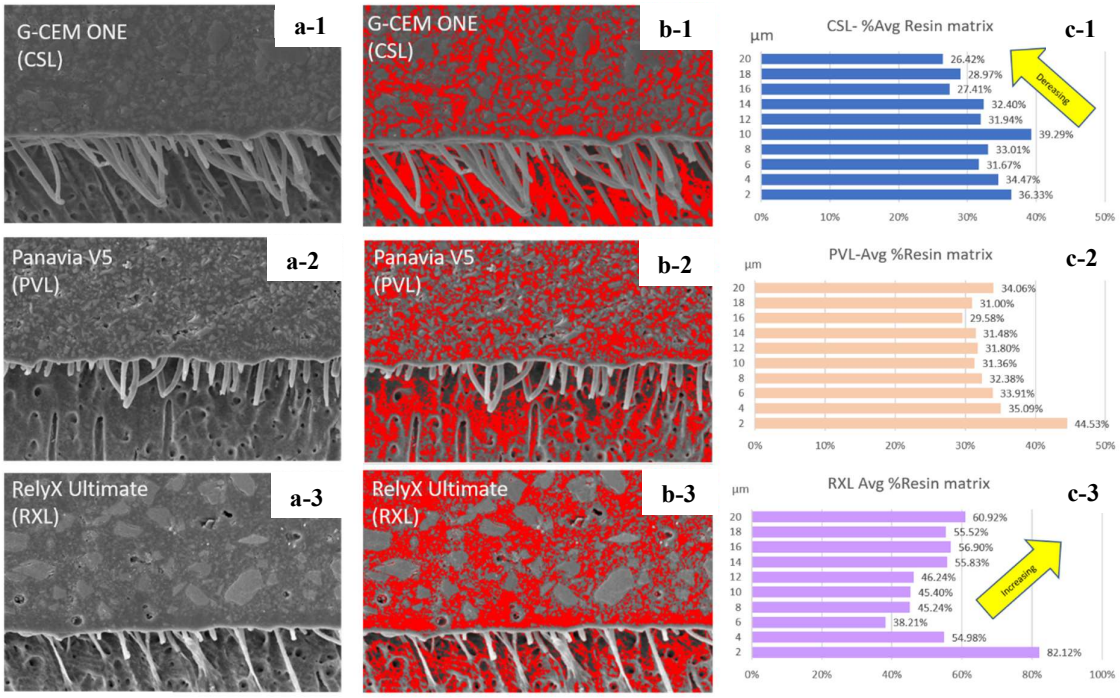


Figure 20 SEM images of the resin-dentin specimen, before, a, and after used Image J software to analyze the amount of resin matrix.

a) Representative SEM images of resin cement were analyzed for % resin matrix from the resin-dentin interface until 20 µm distance in height. CSL, PVL, and RXL are shown as a-1, a-2, and a-3, respectively.
b) The red area represented the SEM images from a (1-3) were analyzed for %resin matrix. Image J software was used for adjusting the threshold, the square-shaped, size 2 x 2 µm, using for each area measurement calculation. The low-value pixels represent the resin matrix became red, while the high-value pixels represent resin filler was not changed. 50 squares of 2x2 µm area were used for analysis for each SEM images.
c) According to Figure 20b, the bar charts show averages % ratio of resin matrix to resin fillers of each cement in each 2-micrometer interval from the resin-dentin interface until 20 micrometers.

Chapter 4: Discussions

The μTBS in this study, for light condition (L-condition), there was no significant difference ($p < 0.05$) regardless of the cements. In contrast, regarding the dark condition (D-condition), the significant difference was found among all cements, $p < 0.05$ for CS-D and PV-D, and $p < 0.001$ for CS-D and RX-D. Only one of "Touch and cure" type resin cement, CS, in which the μTBS was not affected by the light condition. While for the conventional type dual-cure resin cement, RX, the μTBS was significantly decreased ($p < 0.001$). Therefore, the null hypothesis is rejected.

Our study showed the same μTBS results as previous studies, in which the dark condition did not affect "Touch and cure" type resin cement while the μTBS of dual-cure resin cement was decreasing. [8–10,20,47] In this study, we try to minimize the amount of light when testing the D- condition in the darkroom, which would probably similar to the clinical condition under high opaque zirconia or metal indirect restoration that light can not pass through.

Our result also shows μTBS of RX, RelyX Ultimate, was dramatically reduced by 88% when testing in the darkroom D-condition, comparing to the L- condition. This massive reduction may because the μTBS depends on the two-fold mechanism, light-curing, and chemical-curing of resin cement. Previous studies showed that the degree of conversion of dual-cured resin cement achieved by light-activated is higher than chemical activated. [7] [20]

In dark condition, in case of RX when using Scotchbond Universal as an adhesive, there is no light for activating the initiator of the polymerization reaction, thus probably the free radical would come from mixed cement only, and this limitation of free radial might impair the efficiency of the degree of conversion. Consequently, μTBS was significantly reduced.

In contrast, the “Touch and cure” type dual-cured resin cement, CS and PV, they contain catalyst in both primer and cement. As a result, the reaction starts when their primer contact with the cement. This process is the so-called “Touch and Cure” system. [10] Thus in dark condition, this touch and cure system might occur.

The nanoindentation hardness test is a useful test to measure the polymerization surface hardness that can be used to evaluate the degree of conversion of the material indirectly. The hardness values were determined based on both elastic and plastic deformations. The differences in resin phase modulus or in filler types could cause the hardness develops differently. From our results, CSL at 72 h showed the highest hardness value among all groups, and only this cement’s hardness was significantly increased over time ($p < 0.05$).

This may probably because of the different material composition and filler size and distribution pattern or quality of polymerization reaction.

Based on our results, they seem that even the companies claimed that the new reactor was added into either primer or cement and expecting for more increasing chemical reaction, as “Touch and cure” (CSL and PVL), compared to conventional type dual-cured resin cement (RXL). However, our results show only not all of the “Touch and cured” type can provide higher μTBS or DC than conventional type ducal-cured resin cement. Therefore, we assume that there are more other compositions in resin cement might also influence DC, which will influence the μTBS. For example, the different of resin filler distribution and its effect on the light transmission ability, through the cement content, e.g., optical property as demonstrated in this study, since the amount of light is essential for polymerization reaction in dual-cured resin cement.

When deeply investigate the detail of mixed cement in the submillimeter close to the resin-dentin interface, the composition of resin cement should be considered. As previously

explained, the main composition of resin cement is resin filler and resin matrix. However, the compositions of resin cement are various, complicated, and different among each cement. In the aspect of resin fillers, they are various in percentages of fillers by volume or type of filler. In addition to the resin matrix, for example, the type of containing resin monomer or the initiator type used is different. Therefore, we should not focus only on which type of new initiator is used and expected for enhancing polymerization because all of these resin cement composition factors could affect the material properties; hardness and DC, as we investigated in this study.

Firstly, for the resin matrix, during the polymerization process, the monomer reacts to form the polymer. The molecular distance will reduce. The mobility of the monomer-chain can be restricted either by the high volume amount of filler or the type and ratio of high viscous monomer. The higher the viscous monomer contains, the slower down monomer molecule mobility occurs. Polymerization will be more difficult to arise and lead to lower %DC as PVL and RXL result in this study.

Both RXL and PVL contains TEGDMA, which combines with BisGMA, while the only CSL contains UDMA. Our result was the same as the previous study. [31] but different from the Gajewski VES et al. in 2012 [48] in which they found TEGDMA monomer showed the highest DC, followed by UDMA and BisGMA. These may explain by the different materials used. In the study of Gajewski VES et al. [48], they compared the tested homopolymer resin-based dental composites in terms of their DC, while our study tested the commercial resin cements. These marketed resin cements are composed of one or more methacrylate base monomer that each component may affect each other. Furthermore, the monomer content and ratio in our commercially tested cement is not described in the composition provided by the manufacturer.

Even though both PVL and RXL contain TEGDMA, but only PVL also contains Bis-GMA, which is a high molecular weight monomer and viscosity. These characteristics could hinder the monomer molecule mobility and leads to lower conversion, as described before. Therefore, even if PVL is “Touch and cure” dual-cured resin cement type, which its % DC should higher than RXL, conventional dual-cured type, our study indicated PVL and RXL showed almost same DC result. In contrast, the DC of PVL was significantly inferior in both 3 and 72 h than CSL although both cements are “Touch and cure”.

Secondly, for the resin filler aspect, we would like to discuss both the percentage of resin filler distribution and the filler type. In this study, we analyzed the resin matrix instead of resin filler distribution because the monomer area in SEM images is more suitable to calculate with our previously explained method using ImageJ software. However, these percentages of resin matrix distribution can convert into filler distribution, which commonly used for evaluating the material properties by subtraction from 100%.

The resin matrix distribution (Figure 20-1 and Figure 20-2 a, b and c), was observed at approximately 1 mm from the interface both CSL and PVL showed the “liquid-rich zone” (Figure 19-b 1 and 2) Almost same as RXL, but this is cement shows the high values in RXL group demonstrated at the 2- and 4-um data (Figure 20-3 a, b and c), which we assume that area probably is the thickness of adhesive that thicker than those of CSL and PVL. This adhesive layer can be observed by the SEM images (Figure 19-b 3). As both CSL and PVL primer thickness are shown in TEM (Figure 9) and SEM images (Figure 19-b), these primer layers are very thin, approximate 1 mm.

The % of filler distribution might also affect the % DC in the aspect of the capability of monomer molecule mobility, as previously explained.

Another factor regarding the resin fillers are considered would be the filler type. As the resin filler can enhance or retard, due to the difference ability in the scattering effect of light source crossing through resin cement depends on its amount and type. Besides, all three resin cements in this study are dual-cured type and they can able to start the reaction with light-activated or together with the chemical activation. However, various studies show that when dual-cure resin cement was activated with light, their mechanical properties, such as μTBS, hardness, or degree of conversion, are higher when compare with those that are performed under light condition was limited. [20,49] This can clearly see from the SEM images the homogenous distribution of PVL filler shows the same trend as %DC distribution in each interfacial distant; no correlation, from this reason we assume that the light may distribute homogeneously from the resin interface, according to the presentation of the resin filler.

In the current study, the RXL, group, the adhesive, RelyX Universal, was not light-cured before building up the RelyX Ultimate. SEM image of resin-dentin of this cement showed the adhesive layer was approximately 4-5 μm that was thicker than CSL and PVL which were approximately 1 μm. (Figure b1-3) The thickness of RXL in this study is almost same as the study of Araoka et al. [50], which they found that when light-curing RelyX Ultimate adhesive, the thickness is 5-10 μm. Due to the thicker of the adhesive layer may less susceptible to the oxygen [51], consequently the polymerization reaction was less affected. As a result, even though RXL is a conventional type of dual cured resin cement but its DC was not different from PVL, one of the “Touch and cure” type dual cured resin cement.

Before testing, we speculate that both results, μTBS and DC, of RXL, should be inferior to those two “Touch and cured” type, due to that the company recommends to light cure the adhesive for the best performance but our study designed not to light-cure the adhesive before cement application for closely simulate the clinical situation. However, our μTBS and DC results showed the performance of RXL was similar to one of “Touch and cured,” PVL,

which the primer can polymerize under dual-cured mode. These findings may explain by the chemical composition, the thickness of resin base material is also influencing the performance. By the evidence that the thinner the adhesive layer, the more susceptible to inhibit polymerization process by oxygen [52], however, RXL adhesive thickness is approximate 4-5 µm in SEM images (Figure 19b). This layer thickness may adequate to withstand oxygen inhibition in the adhesive layer. Therefore alternative hypothesis is partially accepted.

Two more chemical compositions of resin-based material that could influence DC are HEMA and type of photo-activator. HEMA, a low-molecular-weight monomer that is frequently used in adhesives for the positive effect on the bond strength and type of photo-activator, also affects DC. Van Landuyt et al. (2008) [53] explain the role of HEMA in one step self etch adhesive, the same classification as used for conditioning dentin before mixed cement application. Their study revealed the phase separation detected in uncured one-step self etch adhesives and mention HEMA may adversely affect the polymerization reaction because of retained water results in lower DC. In our study, Panavia V5 primer and Scotchbond Universal adhesive contain HEMA, while G CEM ONE is HEMA free.

For the chemical initiator, generally, camphorquinone (CQ) is combined with tertiary amine and acts as a co-initiator used in the light-activated resin material. Recently an alternative photoinitiator to CQ is diphenyl(2,4,6 trimethyl benzoyl)phosphine oxide (TPO) has been introduced. This photoinitiator can use without co-initiator, e.g., amine, to overcome the neutralized tertiary amine by the acid-based reaction. This might be the advantage of TPO over CQ results in higher DC compare to CQ. [53]

One available TPO-based material is G-Premio BOND (GC, Japan), which are tested cement, G CEM ONE, adopts this technology, and combines with a unique chemical polymerization

initiator. The company's document provides this data. However, they do not clearly show which photoinitiators are using in their cement. It might assume that maybe G-CEM ONE contains TPO as one of the photoinitiators. As a result, it might be one of the reasons for G-CEM ONE; CS, in this study, shows better performance as compared to the other two cement for all tests, μTBS, microhardness, DC. Especially, μTBS of CSL was not reduced under the darkroom.

We also speculated that maybe the good results in terms of μTBS and DC were due to a well-balanced ratio of MDP and Bis-GMA. However, this speculation needs further investigation.

Lastly, it should be taken into consideration when testing "dark condition" or self-cured mode. Although the light was not directly activated from the light-curing unit, however, we speculate that maybe the irradiating lights, from the ceiling light in the experimental room, could influence the result of the dark condition. Consequently, it would not probably reflex the real clinical situation, as we expect, where light is very limited and unable to pass through the metal or very high opaque restoration. Therefore, in this study, the "dark condition" was tested dual-cured resin cement in the self-cure mode in the darkroom under safelight because we would like to minimize the effect from light as much as possible and our observation showed a severely adverse effect on the μTBS of the conventional type dual-cure resin cement.

Conclusion

Within the limitation of this study, the μTBS of "Touch and cure" type dual-cured resin cement is not affected by the dark condition. Both microhardness and DC of "Touch and cure" type dual-cured resin cement, G-CEM ONE, show the highest values among all tested cements in both 3 and 72 h. From the correlation of DC and the distance from resin-dentin interface, it might assume that the touch and cure reaction would probably occur within 15 μm. The DC of resin cement is not influenced by the chemical initiator only, but other compositions both resin matrix and filler also affect the DC.

References

[1] Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265–73.

[2] Rosenstiel SF, Land MF, Crispin BJ. Dental luting agents: A review of the current literature. J Prosthet Dent 1998;80:280–301.

[3] Ferracane JL, Stansbury JW, Burke FJT. Self-adhesive resin cements - chemistry, properties and clinical considerations. J Oral Rehabil 2011;38:295–314.

[4] Farrrell C V., Johnson GH, Oswald MT, Tucker RD. Effect of cement selection and finishing technique on marginal opening of cast gold inlays. J Prosthet Dent 2008;99:287–92.

[5] Fonseca RG, Santos JG dos, Adabo GL. Influence of activation modes on diametral tensile strength of dual-curing resin cements. Braz Oral Res 2005;19:267–71.

[6] Inokoshi M, Pongprueksa P, de Munck J, Naert I, van Meerbeek B V., Minakuchi S, et al. Influence of light irradiation through zirconia on the degree of conversion of composite cements. J Adhes Dent 2016;18:161–71.

[7] Stamatacos C, Simon JF. Cementation of indirect restorations: an overview of resin cements. Compend Contin Educ Dent 2013;34:42–4.

[8] Lührs AK, De Munck J, Geurtsen W, Van Meerbeek B. Composite cements benefit from light-curing. Dent Mater 2014;30:292–301.

[9] Kawano S, Fu J, Saikaew P, Chowdhury AA, Fukuzawa N, Kadowaki Y, et al. Microtensile bond strength of a newly developed resin cement to dentin. Dent Mater J 2015;34:61–9.

[10] Kadowaki Y, Kakuda S, Kawano S, Katsumata A, Ting S, Hoshika S, et al. Bond performance of “Touch and Cure” adhesives on resin core systems. Dent Mater J 2016;35:386–91.

[11] Moosavi H, Hariri I, Sadr A, Thitthaweerat S, Tagami J. Effects of curing mode and moisture on nanoindentation mechanical properties and bonding of a self-adhesive resin cement to pulp chamber floor. Dent Mater 2013.

[12] De Souza GM, Braga RR, Cesar PF, Lopes GC. Correlation between clinical performance and degree of conversion of resin cements: A literature review. J Appl Oral Sci 2015;23:358–68.

[13] Hill E, Lott J. A clinically focused discussion of luting materials. Aust Dent J 2011;56:67–76.

[14] Sokolowski G, Szczesio A, Bociong K, Kaluzinska K, Lapinska B, Sokolowski J, et al. Dental resin cements-The influence of water sorption on contraction stress changes and hydroscopic expansion. Materials (Basel) 2018;11:973.

[15] Asmussen E, Peutzfeldt A. Influence of UEDMA, BisGMA and TEGDMA on selected mechanical properties of experimental resin composites. Dent Mater 1998;14:51–6.

[16] Caughman WF, Chan DC, Rueggeberg FA. Curing potential of dual-polymerizable resin cements in simulated clinical situations. J Prosthet Dent 2001;86:101–6.

[17] Kumbuloglu O, Lassila LVJ, User A, Vallittu PK. A study of the physical and chemical properties of four resin composite luting cements. Int J Prosthodont 2004;17:357–63.

[18] Braga RR, Cesar PF, Gonzaga CC. Mechanical properties of resin cements with different activation modes. J Oral Rehabil 2002;29:257–62.

[19] El-Badrawy WA, El-Mowafy OM. Chemical versus dual curing of resin inlay cements. J Prosthet Dent 1995;73:515–24.

[20] Lührs AK, Pongprueksa P, De Munck J, Geurtsen W, Van Meerbeek B. Curing mode affects bond strength of adhesively luted composite CAD/CAM restorations to dentin. Dent Mater 2014;30:281–91.

[21] Lee IB, An W, Chang J, Um CM. Influence of ceramic thickness and curing mode on the polymerization shrinkage kinetics of dual-cured resin cements. Dent Mater 2008;24:1141–7.

[22] Jang Y, Ferracane JL, Pfeifer CS, Park JW, Shin Y, Roh BD. Effect of insufficient light exposure on polymerization kinetics of conventional and self-adhesive dual-cure resin cements. Oper Dent 2017;42:E1–9.

[23] Shadman N, Atai M, Ghavam M, Kermanshah H, Ebrahimi SF. Parameters affecting degree of conversion of dual-cure resin cements in the root canal: FTIR analysis. J Can Dent Assoc 2012;78:c53.

[24] Tagami A, Takahashi R, Nikaido T, Tagami J. The effect of curing conditions on the dentin bond strength of two dual-cure resin cements. J Prosthodont Res 2017;61:412–8.

[25] Hasegawa EA, Boyer DB, Chan DC. Hardening of dual-cured cements under composite resin inlays. J Prosthet Dent 1991;66:187–92.

[26] Rueggeberg FA, Hashinger DT, Fairhurst CW. Calibration of FTIR conversion analysis of contemporary dental resin composites. Dent Mater 1990;6:241–9.

[27] Navarra CO, Cadenaro M, Frassetto A, Fontanive L, Di Lenarda R, Breschi L. Degree of conversion of self-etch adhesives: In situ Micro-Raman analysis. Oper Dent

2016;41:501–10.

[28] Pianelli C, Devaux J, Bebelman S, Leloup G. The micro-Raman spectroscopy, a useful tool to determine the degree of conversion of light-activated composite resins. J Biomed Mater Res 1999;48:675–81.

[29] Bolaños-Carmona V, Benavides-Reyes C, González-López S, González-Rodríguez P, Álvarez-Lloret P. Influence of Spectroscopic Techniques on the Estimation of the Degree of Conversion of Bulk-fill Composites. Oper Dent 2019:18-095–L.

[30] Schneider LFJ, Consani S, Ogliari F, Correr AB, Sobrinho LC, Sinhoreti MAC. Effect of time and polymerization cycle on the degree of conversion of a resin composite. Oper Dent 2006;31:489–95.

[31] Fonseca ASQS, Labruna Moreira AD, de Albuquerque PPAC, de Menezes LR, Pfeifer CS, Schneider LFJ. Effect of monomer type on the C=C degree of conversion, water sorption and solubility, and color stability of model dental composites. Dent Mater 2017;33:394–401.

[32] Di Francescantonio M, Aguiar TR, Arrais CAG, Cavalcanti AN, Davanzo CU, Giannini M. Influence of viscosity and curing mode on degree of conversion of dual-cured resin cements. Eur J Dent 2013;7:81–5.

[33] Par M, Gamulin O, Marovic D, Klaric E, Tarle Z. Raman spectroscopic assessment of degree of conversion of bulk-fill resin composites-changes at 24 hours post cure. Oper Dent 2015;40:E92–101.

[34] Navarra CO, Breschi L, Turco G, Diolosà M, Fontanive L, Manzoli L, et al. Degree of conversion of two-step etch-and-rinse adhesives: In situ micro-Raman analysis. J Dent 2012.

[35] Shin TP, Yao X, Huenergardt R, Walker MP, Wang Y. Morphological and chemical characterization of bonding hydrophobic adhesive to dentin using ethanol wet bonding technique. Dent Mater 2009;25:1050–7.

[36] Lise DP, Van Ende A, De Munck J, Yoshihara K, Nagaoka N, Cardoso Vieira LC, et al. Light irradiance through novel CAD–CAM block materials and degree of conversion of composite cements. Dent Mater 2018;34:296–305.

[37] Miletic V, Santini A, Trkulja I. Quantification of monomer elution and carbon–carbon double bonds in dental adhesive systems using HPLC and micro-Raman spectroscopy. J Dent 2009;37:177–84.

[38] Hass V, Luque-Martinez I, Sabino NB, Loguercio AD, Reis A. Prolonged exposure times of one-step self-etch adhesives on adhesive properties and durability of dentine bonds. J Dent 2012;40:1090–102.

[39] Hass V, Cardenas A, Siqueira F, Pacheco R, Zago P, Silva D, et al. Bonding Performance of Universal Adhesive Systems Applied in Etch-and-Rinse and Self-Etch Strategies on Natural Dentin Caries. Oper Dent 2019;44:510–20.

[40] Araoka D, Hosaka K, Nakajima M, Foxton R, Thanatvarakorn O, Prasansuttiporn T, et al. The strategies used for curing universal adhesives affect the micro-bond strength of resin cement used to lute indirect resin composites to human dentin. Dent Mater J 2018;37:506–14.

[41] Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTBS) approach. Dent Mater 2017;33:133–43.

[42] Van Landuyt KL, De Munck J, Mine A, Cardoso M V., Peumans M, Van Meerbeek B. Filler debonding & subhybrid-layer failures in self-etch adhesives. J Dent Res 2010;89:1045–50.

[43] Ferracane JL, Hilton TJ, Stansbury JW, Watts DC, Silikas N, Ilie N, et al. Academy of Dental Materials guidance—Resin composites: Part II—Technique sensitivity (handling, polymerization, dimensional changes). Dent Mater 2017;33:1171–91.

[44] Sagadevan S, Murugasen P. Novel Analysis on the Influence of Tip Radius and Shape of the Nanoindenter on the Hardness of Materials. Procedia Mater Sci 2014;6:1871–8.

[45] Chowdhury AFMA, Saikaew P, Matsumoto M, Sano H, Carvalho RM. Gradual dehydration affects the mechanical properties and bonding outcome of adhesives to dentin. Dent Mater J 2019;38:361–7.

[46] Dahlman L. Part 4—Measure Areas using Thresholding. Meas Distance Area Satell Images 2011. https://serc.carleton.edu/eet/measure_sat2/part_4.html (accessed November 18, 2019).

[47] Katsumata A, Saikaew P, Ting S, Katsumata T, Hoshika T, Sano H NY. Microtensile Bond Strength Bonded to Dentin of a Newly Universal Adhesive. J Oral Tissue Eng 2017;15:18–24.

[48] Gajewski VES, Pfeifer CS, Fróes-Salgado NRG, Boaro LCC, Braga RR. Monomers used in resin composites: Degree of conversion, mechanical properties and water sorption/solubility. Braz Dent J 2012;23:508–14.

[49] Tagami A, Takahashi R, Nikaido T, Tagami J. The effect of curing conditions on the dentin bond strength of two dual-cure resin cements. J Prosthodont Res 2017;61:412–8.

[50] Araoka D, Hosaka K, Nakajima M, Foxton R, Thanatvarakorn O, Prasansuttiporn T, et al. The strategies used for curing universal adhesives affect the micro-bond strength of resin cement used to lute indirect resin composites to human dentin. Dent Mater J 2018;37:506–14.

[51] Kim J-S, Choi Y-H, Cho B-H, Son H-H, Lee I-B, Um C-M, et al. Effect of light-cure time of adhesive resin on the thickness of the oxygen-inhibited layer and the microtensile bond strength to dentin. J Biomed Mater Res Part B Appl Biomater 2006;78B:115–23.

[52] Rueggeberg FA, Margeson DH. The Effect of Oxygen Inhibition on an Unfilled/Filled Composite System. J Dent Res 1990;69:1652–8.

[53] Pongprueksa P, Miletic V, Janssens H, Van Landuyt KL, De Munck J, Godderis L, et al. Degree of conversion and monomer elution of CQ/amine and TPO adhesives. Dent Mater 2014;30:695–701.