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Comparative Evaluation of Microtensile Dentin Bond Strength and Interfacial Micromorphology of Three Universal Adhesives

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ABSTRACT

Objectives: To assess and compare the microtensile dentin bond strength (µTBS) and interfacial micromorphology of three universal adhesives. Methods: 96 human molars were assigned to three universal adhesives: Single Bond Universal (SBU), CLEARFIL Universal Bond Quick (UBQ), and RE-GEN Universal Adhesive (REGEN). Adhesives were applied in self-etch mode. SBU and REGEN were applied following the manufacturers' instructions. UBQ was divided into two subgroups: one following the manufacturer's instructions (UBQ Short) and the other with an extended application time (UBQ Extended). Teeth were restored with nanohybrid resin composite. Specimens were divided into immediate and delayed subgroups. The delayed subgroups were stored for 6 months and subjected to 5000 thermocycles. µTBS was tested, and failure mode was analyzed. Interfacial micromorphology was assessed using a scanning electron microscope. The data were statistically analyzed ($p < 0.05$). Results: The adhesive choice, aging, and their interaction significantly affected µTBS. SBU exhibited the highest immediate µTBS, comparable to UBQ (Extended) and REGEN, and significantly higher than UBQ (Short). In delayed testing, SBU outperformed the other adhesives. Conclusions: Aging negatively affected the µTBS of UBQ and REGEN, while SBU wasn't affected. The quick application concept of UBQ deteriorated its µTBS compared to the extended application time.

INTRODUCTION

Universal adhesives are the latest member in dental adhesive systems. They provide stable and reliable bonding to different substrates due to their specific functional monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP),[1] which interact strongly with calcium ions in the hydroxyapatite (HAp) of the tooth.[2] It causes minor decalcification of HAp, resulting in release of Ca and formation of stable MDP-Ca salts (nanolayering), providing both chemical and micromechanical adhesion of universal adhesives and increasing their resistance to degradation.[3] There are continuous improvements to universal adhesives to optimize their properties and their application to different dental substrates while maintaining bond durability.[4]

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Some of these enhancements include fluoride-releasing adhesives, which are expected to promote the mineralization of dental tissues around composite restorations, reducing secondary caries and increasing the longevity of the restorations.[5] Currently, the ideal dental material should be biocompatible, biomimetic, and bioactive.[6] There have been continuous experimental and commercial trials to obtain bioactive dental adhesives.[7,8] However, there are few data in the literature about the available commercial universal adhesives containing bioactive glasses. Another type of improvements made by some manufacturers to simplify the application of universal adhesives is the quick application concept, which suggests just applying the adhesive and light curing, with no waiting time.[9]

Despite the advancements in dental adhesives, it is still crucial to ensure a reliable and stable bond. Achieving a predictable bond strength directly impacts the effectiveness of dental adhesives, which determines the success or failure of dental composite restorations. The microtensile bond strength test is the most commonly used method to evaluate the bonding performance of dental adhesives.[10] Simultaneously, the micromorphological evaluation enables a thorough analysis of the adhesive-substrate interface, revealing any potential interface interactions that may influence the long-term stability of the bond. Consequently, the microtensile bond strength test, along with micromorphological evaluation, is widely recognized as a standardized and versatile method for assessing the bonding performance of adhesives to dental substrates.[10] The adhesion between enamel and adhesive resin is strong and predictable, so enamel adhesion has been considered reliable. However, the heterogeneous nature of dentin and the clinical situations make dentin bonding challenging and less predictable.[10]

This study investigated the effect of some improvements in universal adhesives on dentin bond durability and the micromorphology of the adhesive/dentin interface using three different universal adhesives. The null hypotheses tested were that (1) there would be no statistically significant difference in μTBS among the evaluated adhesives, (2) the storage and thermocycling would not statistically affect the μTBS and the interfacial micromorphology of each of the evaluated adhesives, and (3) the application time would have no statistically significant effect on the μTBS of UBQ.

METHODS

Three different universal adhesives (UAs) and one nanohybrid resin composite were used in this study. Table 1 illustrates the details and manufacturers' instructions for use of the tested materials.

TEETH SELECTION AND PREPARATION

96 sound human upper permanent molars extracted following the Ethical Committee's regulations, with approval number: A01041022, were collected. Teeth were cleaned, polished, and stored in 0.5% chloramine-T for 24 hours at 4 ºC.[11] Then, teeth were stored in a weekly-changed distilled water at 4 ºC for 3 weeks.[12] Teeth were numbered and randomly assigned to SBU, UBQ, and REGEN groups, with UBQ further divided into short and extended subgroups.

Each tooth was mounted in acrylic resin inside a cylindrical polyvinyl chloride tube. A proximal enamel slice was removed. Midcoronal dentin was identified beyond the amelodentinal junction (ADJ) by 1–1.5 mm, then exposed using a diamond cutting disc mounted in an IsoMet 4000 Linear Precision Saw (Buehler, Lake Bluff, IL, USA) under copious water perpendicular to the long axis of the tooth. Teeth were examined for pulp exposure or enamel remnants under a stereomicroscope (Nikon SMZ745T, Nikon, Tokyo, Japan). A standardized smear layer was created using Al_2O_3 finishing discs in a low-speed handpiece under copious water.

BONDING PROTOCOL AND COMPOSITE APPLICATION

All bonding and restoration procedures were applied by the same operator to reduce variability. After smear layer preparation, adhesives were applied in the self-etch (SE) application mode, following the manufacturers' instructions *(Table 1)*. In UBQ (Extended), the same manufacturer's steps were applied except for rubbing the adhesive for 20 secs. Adhesives were light-cured with an LED light curing unit; 3M ESPE Elipar (3M Deutschland, Neuss, Germany), with a radiant exitance of 1200 mW/cm^2. Bonded dentin surfaces were incrementally built up with a nanohybrid resin composite; Tetric N Ceram. The curing unit was calibrated and regularly checked by a radiometer.

MICROTENSILE BOND STRENGTH (μTBS) TESTING AND FAILURE MODE ANALYSIS

80 teeth were randomly selected for the μTBS test: 20 teeth from each adhesive group. The teeth of each adhesive group were subdivided into immediate and delayed subgroups (n = 10). Delayed subgroups were stored in artificial saliva at 37 °C for 6 months and then subjected to thermocycling for 5000 cycles between 5 ± 2°C and 55 ± 2°C. 13 The dwell time was 30 secs[13] and the transfer time was 5 secs.[14] Teeth of the immediate subgroups were cut using a diamond cutting disc mounted in an IsoMet 4000 (Buehler, USA) at 2550 rpm under copious water cooling and evaluated after 24 hours of storage in artificial saliva. Six central beams (1 mm x 1 mm) from each tooth were prepared and measured for μTBS testing using a universal testing machine (Instron, model 3345, MA, USA) with a 1.0 N tensile load and a 1 mm/min loading rate until debonding.[15] Results were recorded in MPa. μTBS

Table 1. Materials used in the study, their compositions, and their application steps according to their manufacturers' instructions.

Material	Manufacturer (website) & lot number	Solvent	Components	Instructions for application
Single Bond Universal Adhesive (SBU) **(pH=2.7) Mild**	3M Deutschland GmbH, Carl-Schurz-Straße, Neuss, Germany (www.3MESPE.comwww.3MESPE.com) # ********707	Ethanol/water-based solvent system	10-MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler (nanosilica), Ethanol, Water, Initiators, Silane	Apply the adhesive to the prepared moist dentin. Rubbing the adhesive for 20 secs. Gentle air drying for 5 secs. Apply second coat of the adhesive (Repeat the steps from 1-3). Light cure for 10 secs.
CLEARFIL Universal Bond Quick (UBQ) **(pH=2.3) Mild**	Kuraray Noritake Dental Inc., Kurashiki, Okayama, Japan (www.kuraraydental.comwww.kuraraydental.com) # 160336	Ethanol/water-based solvent system	10-MDP, Bis-GMA, HEMA, Hydrophilic amide monomers, Colloidal silica, Silane coupling agent, Sodium fluoride, DLCamphorquinone, Ethanol, Water	Apply the adhesive to the prepared moist dentin. Rubbing the adhesive for 3 secs only (no waiting). This step was applied only in (UBQ Short) group, while in (UBQ Extended) group, rubbing was for 20 secs (extended application time). Blowing mild air stream for 5 secs. Apply second coat of the adhesive (Repeat the steps from 1-3) Light cure for 10 secs.
Clean & Boost® gel **(Dentin and enamel cleanser gel)**	VISTA I APEX, Inter-Med Inc., Racine, WI, USA (https://www.vistaapex.comhttps://www.vistaapex.com) #02182022	-------	2-hydroxyethyl methacrylate, Propanol, Nitric acid	Apply Clean & Boost® gel to the prepared moist dentin using the Stat-Flo delivery brush tip (VISTA I APEX, # 21101) until complete saturation. With the delivery brush tip, agitate the Clean & Boost® gel on the surface for 10 seconds. Rinse thoroughly for 20 secs. Gentle air drying for 5 secs before applying REGEN adhesive.
RE-GEN* universal adhesive (REGEN) **pH: No data available**	VISTA I APEX, Inter-Med Inc., Racine, WI, USA (https://www.vistaapex.comhttps://www.vistaapex.com) # 20221678	Ethanol-based solvent system	Bioglass 45S5**, Bisphenol A glycerolate dimethacrylate (Bis-GMA) (20-25%), Ethyl alcohol (18-22.5%), Pyromellitic dimethacrylate (PMGDM) (15-20%), HEMA (10-15%), 10-MDP, Ethyl 4-dimethylaminobenzoate ***	Applied after thorough rinsing and gentle air drying of Clean and Boost cleanser gel. Dispense one drop of the adhesive into a mixing well and stir thoroughly. Apply one coat of the adhesive to the moist dentin. Allow to dwell for 20 seconds. DO NOT DRY. Apply 2 additional coats of the adhesive. Simultaneous gentle and thorough air drying of the surface for 5 secs. Light cure for 10 secs.
Tetric N Ceram (Nanohybrid resin composite, Shade A2)	Ivoclar Vivadent, Schaan, Liechtenstein (https://www.ivoclar.com/en_lihttps://www.ivoclar.com/en_li) #Z03H1S	-------	Matrix: Dimethacrylates (Bis-GMA, UDMA, TEGDMA) Filler: Barium glass, ytterbium trifluoride, mixed oxides and copolymers	Applied after adhesive application up to 4.5 mm in 3 horizontal increments (each 1.5 mm in thickness). Each increment was light-cured for 20 secs. Additional light cure for 10 secs for each side.

10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate, Bis-GMA: Bisphenol A diglycidylmethacrylate, HEMA: 2-Hydroxyethyl methacrylate, UDMA: Urethane-dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate, PMGDM: Pyromellitic dianhydride glycerol dimethacrylate, Ethyl 4-dimethylaminobenzoate: Benzoic acid, 4-(dimethylamino)-, ethyl ester. * The information about Regen composition was mainly obtained from its Material Safety Data Sheet (MSDS) published by the manufacturer. ** Bioglass 45S5 was mentioned in the VISTA APEX. REGEN sell sheet. In. Racine (USA): VISTA APEX; 2021. https://www.vistaapex.com. * Acetone was included in the composition in the first draft of MSDS (2020) but not mentioned in the revised draft published June 2021.**

procedures followed the guidelines of the Academy of Dental Materials.[16] The test was performed by an experienced operator blinded to the restorative procedures. After deboning, all fractured sites were examined under a stereomicroscope. Fracture types were determined and photographed. Failure modes were either adhesive (A), cohesive on composite (C.C), cohesive on dentin (C.D), or mixed (M).[13]

SPECIMEN PREPARATION FOR SEM ANALYSIS OF THE INTERFACIAL MICROMORPHOLOGY

Two teeth from each immediate and delayed subgroup were sectioned using a diamond cutting disc mounted in IsoMet under water cooling parallel to the long axis of the tooth, then horizontally 1 mm below CEJ to obtain two similar halves. Each half was polished under water cooling with sequenced grits of SiC_2 papers (600–4000), each for 20 secs, and rinsed to remove debris. Then, each half was polished using fine diamond pastes and cleaned in an ultrasonic cleanser for 10 minutes. For the acid-base challenge, specimens were immersed in saline for 10 minutes, then in a 10% orthophosphoric acid solution for 10 secs, and in a 5% sodium hypochlorite solution for 5 minutes, respectively, then water rinsed for 15 secs, and left to be completely dry for 24 hours at 37 ºC.[17] Specimens were gold-sputtered (SPI Module-Sputter Coater System, EDEN Instruments, Valence, France) and examined under a scanning electron microscope (SEM) (JSM-6510 LV, JEOL, Japan) at magnifications of x500, x1000, and x2000. Different areas were examined along the resin/dentin interface of each specimen. At x1000 and x2000, the thickness of adhesive layers (AL) and hybrid layers (HL) and the depth range of the most apparent resin tags (RTs) into dentinal tubules (DTs) were measured in µm.

STATISTICAL METHODS

Sample size calculation

In this study, the sample size for the µTBS test was calculated using the statistical G*Power program (G*Power Ver. 3.1.9.7, Kiel, Germany). Ten samples in each subgroup (n = 10) achieved an effect size (f) = 1.2, with an 80% statistical power (type II error) and a 0.05 type I error (α).

STATISTICAL ANALYSIS

An investigator blinded to the evaluated groups analyzed the collected µTBS values using Statistical Package for Social Science (SPSS), version 20 (IBM, NY, USA). Test of normality (Kolmogorov-Smirnov test) and homogeneity of variances test (Levene's test) proved the normal data distribution of the µTBS values between groups. So, two-way analysis of variances (ANOVA) test was used to determine the statistical effects of the adhesive type, aging, and their interaction on the µTBS values. Then, Tukey HSD post-hoc test was used for multiple comparisons among the tested groups with a statistical significance of $p < 0.05$.

RESULTS

MICROTENSILE BOND STRENGTH (µTBS) TEST

Two-way ANOVA test revealed statistically significant effects of the adhesive type, the aging ($p < 0.001$), and their interaction on the µTBS values ($p = 0.038$) *(Table 2)*. According to Tukey HSD post-hoc test results *(Table 3)*, in the immediate subgroups, SBU adhesive had the highest µTBS values, followed by UBQ (Extended) and REGEN, with no statistically

Table 2. Two-way analysis of variance test results.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Aging	1157.323	1	1157.323	61.891	0.000
Adhesive	1530.994	3	510.331	27.291	0.000
Aging * Adhesive	170.294	3	56.765	3.036	0.038

Table 3. Means ± standard deviation of dentin microtensile bond strength (µTBS) in mPa among the adhesive groups tested in this study.

Adhesive	SBU	UBQ (Short)	UBQ (Extended)	REGEN
Immediate	44.2835 ± 5.32 ^{Aa}	34.2541 ± 4.63 ^{Bb}	42.4583 ± 2.64 ^{Aa}	41.0897 ± 4.2 ^{Aa}
Delayed	40.8342 ± 4.35 ^{Aa}	22.0188 ± 3.91 ^{Cc}	33.4967 ± 5.21 ^{Bb}	29.3676 ± 3.72 ^{Bb}

Mean values followed by different superscript uppercase letters indicate significant difference between groups within rows
Mean values followed by different superscript lowercase letters indicate significant difference between groups within columns

significant difference between them ($p > 0.05$). UBQ (Short) obtained the lowest μTBS values with statistically significant differences from the other subgroups ($p < 0.05$). In the delayed subgroups, SBU obtained the highest μTBS values with statistically significant differences from the other subgroups ($p < 0.05$). There was no statistically significant difference between UBQ (Extended) and REGEN adhesives ($p > 0.05$). UBQ (Short) had the lowest μTBS values with statistically significant differences from the other subgroups ($p < 0.05$). For comparison of the μTBS values within each adhesive group, in the SBU adhesive group, there was no statistically significant difference between immediate and delayed subgroups ($p > 0.05$). In the UBQ (Short), UBQ (Extended), and REGEN adhesive groups, there were statistically significant differences between the immediate and delayed subgroups ($p < 0.05$).

FAILURE MODE ANALYSIS

The percentage of pre-testing failures (ptf) didn't exceed 3% of the total number of specimens in all groups. No ptf was detected in the immediate subgroup of SBU adhesive. Table 4 shows the results of failure mode analysis. The adhesive failure (A) was the dominant failure mode in all subgroups, followed by the mixed failure except in the immediate SBU subgroup. The cohesive failure in composite (C.C) was the most recorded failure mode in immediate SBU, followed by the mixed failure. The cohesive failure in dentin (C.D) was the least observed failure mode in all subgroups (1.4%).

SEM ANALYSIS OF THE INTERFACIAL MICROMORPHOLOGY

Figures 1 and 2 show representative SEM photos of the resin/dentin interfaces of immediate and delayed subgroups, respectively, of the evaluated adhesives at magnifications of x500, x1000, and x2000. Table 5 demonstrates the average measurements in μm of the adhesive layer thickness (ALT), hybrid layer thickness (HLT), and the depth range of the most prominent RTs within DTs. Comparing ALT and HLT among the subgroups, immediate REGEN photos showed the thickest AL and HL. Immediate UBQ (Extended) and UBQ (Short) had thinner ALs and HLs, respectively. Among the delayed subgroups, delayed UBQ (Extended) had the thickest AL and HL. The average ALT and HLT of the delayed REGEN and SBU decreased, compared to their immediate counterparts. However, the delayed REGEN exhibited relatively thicker AL but thinner HL than the delayed SBU. While the average ALT and HLT of the delayed UBQ (Short) increased, but still had the lowest ALT and HLT of the delayed subgroups.

For the depth range of RTs, in immediate subgroups, REGEN showed the longest and nearly homogenous RTs, followed by SBU. While UBQ (Short) had a decrease in RT depth. UBQ (Extended) had the shortest resin tags. In the delayed subgroups, the length of RTs generally decreased compared to the immediate subgroups. However, delayed REGEN still had the longest-formed RTs. Delayed SBU and UBQ (Extended) nearly had the same depth range of the formed RTs. While the delayed UBQ (Short) had the shortest RTs among the delayed subgroups.

DISCUSSION

In this study, three universal adhesives were assessed, two of them featuring enhancements: fluoride release in UBQ and bioactive properties in REGEN, as claimed by their manufacturers. So, they have some differences in their compositions that might be related to these improvements, such as hydrophilic amide monomers and sodium fluoride in UBQ and bioglass 45S5 in REGEN. These modifications should be tested to assess if they would affect these adhesives' μTBS compared

Table 4. Percentages of failure mode types within the tested adhesive subgroups.

Adhesive Type	Aging	Adhesive failure	Cohesive failure (composite)	Cohesive failure (dentin)	Mixed failure
SBU	Immediate	13.3%	46.7%	0.0%	40.0%
	Delayed	60.0%	10.0%	5.0%	25.0%
UBQ (Short)	Immediate	46.7%	6.7%	0.0%	46.7%
	Delayed	75.0%	10.0%	0.0%	15.0%
UBQ (Extended)	Immediate	40.0%	20.0%	0.0%	40.0%
	Delayed	55.0%	10.0%	0.0%	35.0%
REGEN	Immediate	66.7%	26.7%	0.0%	6.7%
	Delayed	65.0%	5.0%	5.0%	25.0%
Total of each failure type		54.3%	15.7%	1.4%	28.6%

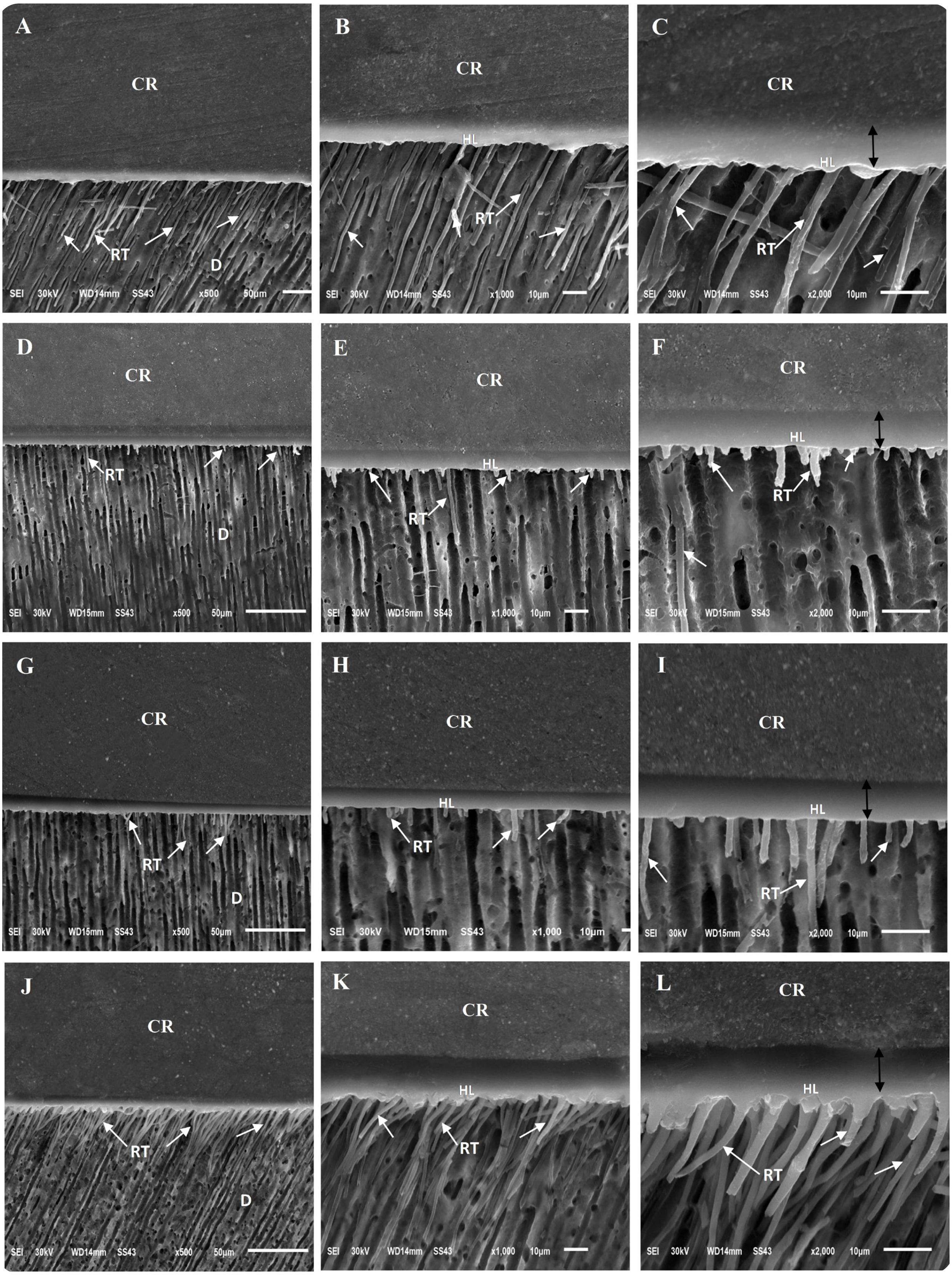

Figure 1: Representative SEM photos of resin/ dentin interfaces of the four immediate subgroups of the evaluated universal adhesives at magnifications x500, x1000, and x2000. CR: composite resin, HL: hybrid layer, RT: resin tags, D: dentin. White arrows point at the formed resin tags. Black doubled arrow refers to the adhesive layer. A, B, and C are representative SEM photos of resin/ dentin interfaces of immediate SBU at x500, x1000, and x2000, respectively. D, E, and F are representative SEM photos of resin/ dentin interfaces of immediate UBQ (Short) at x500, x1000, and x2000, respectively. G, H, and I are representative SEM photos of resin/ dentin interfaces of immediate UBQ (Extended) at x500, x1000, and x2000, respectively. J, K, and L are representative SEM photos of resin/ dentin interfaces of immediate REGEN at x500, x1000, and x2000, respectively.

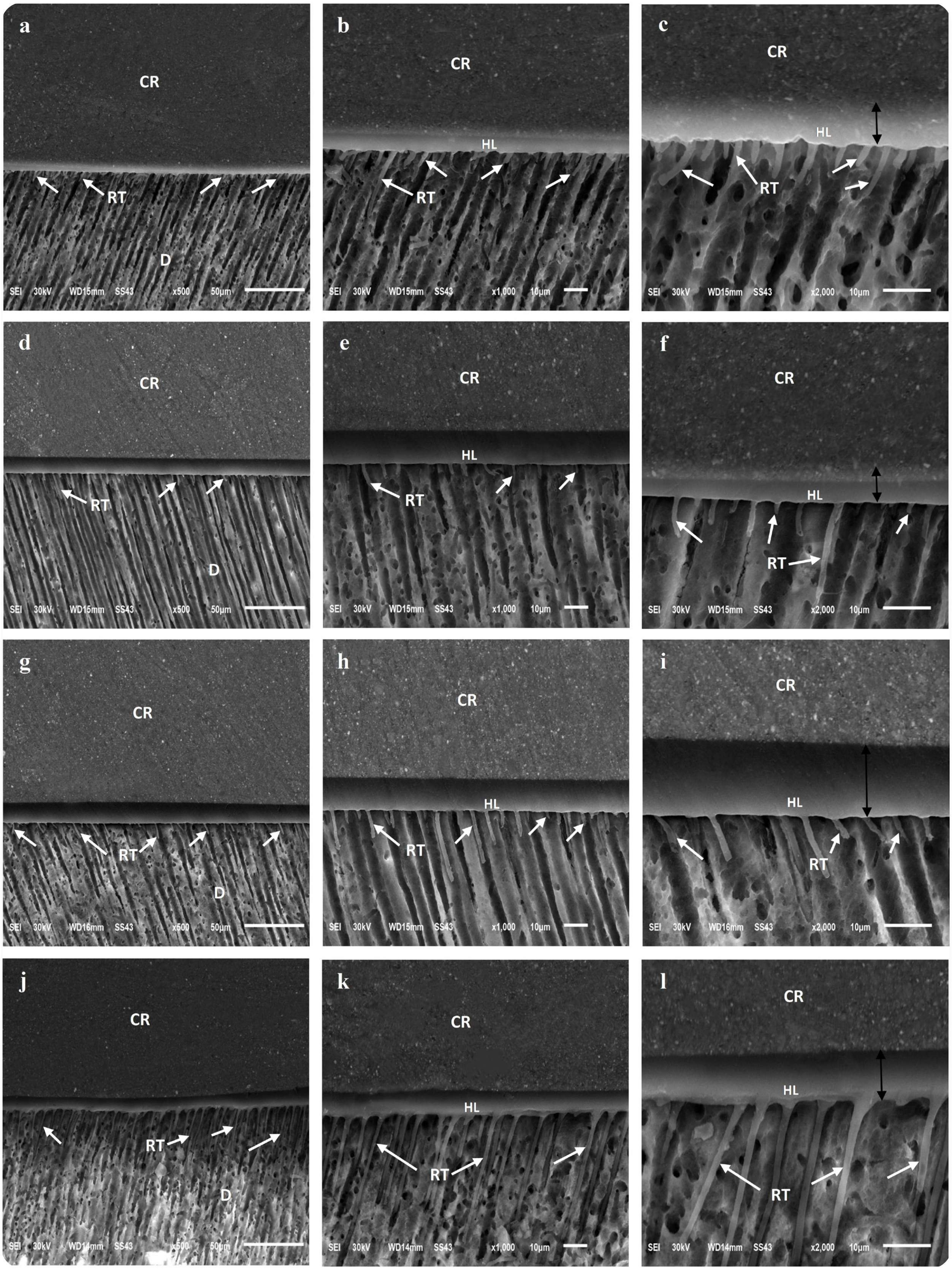

Figure 2: Representative SEM photos of resin/ dentin interfaces of the four delayed subgroups of the evaluated universal adhesives at magnifications x500, x1000, and x2000. CR: composite resin, HL: hybrid layer, RT: resin tags, D: dentin. White arrows point at the formed resin tags. Black doubled arrow refers to the adhesive layer. a, b, and c are representative SEM photos of resin/ dentin interfaces of delayed SBU at x500, x1000, and x2000, respectively. d, e, and f are representative SEM photos of resin/ dentin interfaces of delayed UBQ (Short) at x500, x1000, and x2000, respectively. g, h, and i are representative SEM photos of resin/ dentin interfaces of delayed UBQ (Extended) at x500, x1000, and x2000, respectively. j, k, and l are representative SEM photos of resin/ dentin interfaces of delayed REGEN at x500, x1000, and x2000, respectively.

Table 5. SEM measurements of the average thickness of adhesive layer and hybrid layer and the depth range of resin tags in µm in the evaluated subgroups.

Adhesive	Aging	Adhesive layer (µm)	Hybrid layer (µm)	Depth of resin tags (µm)
SBU	Immediate	7.708	4.935	21.422 – 61.74
	Delayed	7.116	4.702	8.823 – 22.632
UBQ (Short)	Immediate	5.236	3.079	23.618 – 28.823
	Delayed	6.705	3.254	6.6 – 15.55
UBQ (extended)	Immediate	5.4	3.603	15.5 – 24.621
	Delayed	10.512	5.105	10.559 – 21.646
REGEN	Immediate	9.013	5.169	18.14 – 66.219
	Delayed	7.14	4.102	26.776 – 43.768

to another UA (SBU). Consequently, this study didn't specifically evaluate the additional properties of these adhesives; instead, the focus was on assessing the µTBS and examining the adhesive/dentin interface. This choice was due to the utmost importance of the µTBS and interface between the adhesive and dental substrate over the other properties. Even if an adhesive offers innovative properties but compromises its bond strength, those additional properties would be rendered ineffective and less valuable.

A previous meta-analysis found a strong relation between the cohesive failure mode and the high bond strength of adhesives.[18] However, cohesive failure mode might not represent the actual bond strength between adhesive and tooth and it is more related to the dental substrate, resin composite, or test geometry.[19] So, it could be excluded from the statistical analysis of bond strength. However, Franz *et al.*[20] didn't recommend exclusion of data based on a specific fracture mode to avoid bias in the results, as data exclusion would not reflect the actual performance of the strong adhesives. So, in the current study, the recorded µTBS values were included in the statistical analysis without exclusion of cohesive failure specimens to avoid misleading results.

The results of this study indicate that the µTBS was strongly affected by the brand choice of universal adhesives. Artificial aging is thought to affect µTBS tests.[21] Thermocycling of restorative materials is considered the most widely used method in the literature to accelerate the aging process.[21] So, µTBS of each adhesive was evaluated immediately and after storage and thermocycling to detect any significant differences. Deng *et al.*[22] reported that µTBS exhibited its highest values when measured immediately and gradually decreased with storage and thermocycling (i.e., aging). The data of the current study partially support this theory, as the µTBS of three adhesive groups decreased after aging; however, the µTBS of one adhesive group (SBU) wasn't affected.[23] So, the second null hypothesis could be partially accepted.

Based on the µTBS results among adhesives, the first null hypothesis could also be partially accepted, as in the immediate testing, there was no statistically significant difference between SBU, UBQ (Extended), and REGEN adhesives, but they were statistically higher than UBQ (Short) ($p < 0.05$). Also, in the delayed testing, UBQ (Extended) and REGEN were statistically lower than SBU and higher than UBQ (Short). SBU (pH=2.7) obtained the highest and most stable immediate and delayed µTBS values. This is consistent with different previous studies.[15,23,24] This stable µTBS can be attributed to the contribution of several factors: its balanced formula, containing the Vitrebond Copolymer, HEMA, silane, and water, which allows the formation of a consistent hybrid layer and provides high bond strength.[13] Moreover, the Vitrebond Copolymer and 10-MDP monomer allow two chemical bonding mechanisms:[24,25] Vitrebond Copolymer with the hydroxyapatite of the tooth,[26] and the nano-layering formation of 10-MDP monomer with Ca salts.[3] This is in line with the results of previous studies, which reported that adhesives containing Vitrebond Copolymer had higher bond strength than other adhesives.[11,13,23] Moreover, adequate application time and rubbing could contribute to maintaining the bond stability.[27]

REGEN adhesive had a comparable high immediate µTBS to SBU with no statistically significant difference between them. This may be due to its composition with the bioactive glass formula (Bioglass 45S5), claimed by its manufacturer.[28] The manufacturer claimed that the incorporated bioactive glass can attract and exchange ions, such as calcium, phosphate, and fluoride, with the oral environment and enhance hydroxyapatite formation, increasing the bond strength and reducing gap formation at the resin-dentin interface.[29]

In this study, UBQ adhesive was evaluated using two application concepts: no waiting time in UBQ (Short) subgroups, as recommended by the manufacturer, and an extended application time of 20 seconds in UBQ (Extended) subgroups. Based on their results, the third null hypothesis could be rejected as the UBQ (Short) exhibited the lowest immediate and delayed µTBS with statistically significant differences from UBQ (Extended) and from other subgroups. This could be attributed to following the no waiting time concept, which might have been insufficient time and affected its µTBS. Universal adhesives need enough time to infiltrate and hybridize sufficiently to form a stable CaRPO4 structure and enhance bond stability.[27] On the contrary, the UBQ (Extended) had a comparable high immediate µTBS to SBU and REGEN ($p > 0.05$). This finding is in line with previous studies,[13,24,30] where the recommended "no waiting time" protocol was not followed and longer rubbing times were used. The improved immediate µTBS of UBQ (Extended) could be due to the extended application time, allowing for sufficient resin infiltration and hybridization. This is consistent with a previous study by Ahmed *et al.*,[9] which found that a 20-second application time improved the immediate µTBS of UBQ.

For the delayed UBQ (Short), UBQ (Extended), and REGEN subgroups, the bonding performance statistically decreased than its immediate one. This may be due to the slow release of matrix metalloproteinases (MMPs) in dentin, which degrades the collagen fibrils of the hybrid layer over time, gradually decreasing the dentin bond strength, as reported by Pashley *et al.*,[31] Zang *et al.*[32] and Sebold *et al.*[33] UBQ (pH=2.3) has a hydrophilic acrylamide monomer, silane coupling agent, and a reduced HEMA content,[34] which was claimed to decrease water sorption, improve polymerization, and decrease bond degradation with time. However, delayed UBQ (Short) showed a statistically significant decrease in µTBS with aging. This significant decrease agrees with previous studies.[24,35] This may be attributed to the short application time and its solvent of ethanol and water. Ethanol has a low vapor pressure, so it might need more time to evaporate.[24] The residual water[36] and solvent[37] negatively affect polymerization, accelerating hybrid layer degradation over time.[24] Similarly, the µTBS of delayed UBQ (Extended) had a statistically significant decrease than its immediate one. This might be due to its solvent composition. However, the extended application concept statistically improved the aged µTBS of UBQ (Extended) than that of UBQ (Short).

Regarding REGEN, the manufacturer claims that the bioglass formula could improve the long-term bond strength and reduce bond degradation.[29] However, its delayed µTBS was statistically lower than its immediate one. This is likely due to applying successive multiple adhesive layers without air blowing in between, which might cause incomplete evaporation and entrapment of the solvent (ethanol). This residual solvent is thought to affect the polymerization, leading to adhesive/dentin interface degradation over time, as reported by Ikeda *et al.*[37] This has led to a gradual weakening of the µTBS of REGEN with aging.

Irrespective of the adhesive type or aging, adhesive failure between adhesive and dentin was the predominant mode of failure in almost all subgroups, followed by mixed failure,[9,38] except in immediate SBU. The percent of adhesive failure increased with storage and thermocycling in all subgroups. This agrees with previous studies.[9,39] This can be explained by the decrease in µTBS values with aging. The immediate SBU had the lowest adhesive failure and the highest cohesive failure percentage. This could be due to its highest µTBS.[38] Similarly, the delayed UBQ (Short), which had the lowest µTBS, exhibited the highest adhesive failure percentage.[13] However, no general correlation between the failure mode type and µTBS values could be applied to all evaluated subgroups (*Table 4*).

SEM analysis of the immediate subgroups exhibited that REGEN and SBU had the thickest ALs and HLs and the longest RTs. This might be related to their high immediate bond strength. Similarly, the immediate UBQ (Short) had the thinnest ALs and HLs and shorter RTs inside DTs, and it had the lowest immediate bond strength. However, the immediate UBQ (Extended) had thinner ALs and HLs than REGEN and SBU, and the shortest RTs among the immediate subgroups, despite its higher and comparable immediate µTBS to SBU and REGEN. So, the ALT, HLT, and length of RTs are not necessarily indications of the bond strength. This is consistent with previous studies by Kharouf *et al.*,[40] and Rahal *et al.*[41] In the delayed subgroups, SBU had thinner ALs and HLs, with RTs formed at nearly a similar depth range to delayed UBQ (Extended), while it exhibited the highest delayed µTBS among all the delayed subgroups. The homogenous and longest RTs formed in immediate and delayed REGEN could be due to cleaning the dentin surface with Clean and Boost gel before adhesive application, which could facilitate the adhesive penetration into DTs, forming long and uniform RTs.[42]

The current study was focused on assessing the µTBS and examining the resin/dentin interface of the evaluated adhesives and didn't evaluate the improvements made to some of the evaluated adhesives. This preference was because the µTBS and its long-term stability are more crucial factors. As if the adhesives were found to have other great properties but without an adequate µTBS, this would be less valuable. On the contrary, if they proved to have acceptable and stable µTBS, this would recommend doing additional research and testing the other claimed improvements.

According to the results and considering the limitations of this study, additional laboratory studies on UBQ and REGEN are needed to evaluate other properties related to their improvements, such as anti-cariogenic and antibacterial properties. Due to the inherent limitations of laboratory studies, future clinical trials are needed to compare the evaluated adhesives under clinical conditions to validate the clinical performance of composite restorations using these adhesives, which have been concurrently done by the same authors.

For possible future studies, some solutions should be considered. When using the colorless Clean and Boost gel before REGEN adhesive, more rinsing time is needed to ensure its complete removal (20 secs are recommended). Also, the REGEN formula was more fluid, and the components seemed to be a little separable. To overcome this, more bottle shaking before dispensing and thorough mixing before application should be considered.

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CONCLUSIONS

The dentin bond strength of UBQ and REGEN diminished after aging, while SBU was not affected. The quick application of UBQ deteriorated its µTBS, so it is preferable to be used with an extended application time (20 secs).

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