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ORIGINAL RESEARCH

The Role of Duration of Chlorhexidine Gluconate 2% Application on the Shear Bond Strength of a Total Etch Bonding Agent: A Comparative Study

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Introduction: Matrix metalloproteinases enzymes (MMPs) can degrade the hybrid layer which can cause failure of composite restorations. Chlorhexidine gluconate 2% can reduce MMPs activity and increase the bond strength of the resin to dentin.

Purpose: This study aims to determine the role of the duration of chlorhexidine gluconate 2% application on shear bond strength of a total-etch bonding agent.

Methods: A total of 36 freshly extracted maxillary premolars were removed occlusally by one-third of the crown using a carborundum disc until the dentin was exposed. Specimens were divided into four groups n(9). The dentin surfaces were etched for 5s. Group A is the control group. In group B, chlorhexidine gluconate 2% was applied for 30s. In group C, chlorhexidine gluconate 2% was applied for 60s. In group D, chlorhexidine gluconate 2% was applied for 90s. The universal adhesive was applied afterwards and then followed by composite to the dentin surface. All specimens were stored in artificial saliva at 37°C for 24 hours. The shear bond strength was tested using a universal testing machine.

Results: There was an increase in the shear bond strength of the bonding agent along with the additional application duration of chlorhexidine gluconate 2%. All groups gave higher MPa values than the control group. The shear bond strength in group A (control) was 12.64 MPa; Group B (30s of chlorhexidine) was 16.93 MPa; Group C (60s chlorhexidine) was 18.23 MPa; group D (90s of chlorhexidine) was 18.47MPa.

Conclusion: Duration of chlorhexidine gluconate 2% application affects the shear bond strength of the bonding agent with the total-etch system. The effective duration of chlorhexidine gluconate 2% for the restorative procedure is 60 seconds.

Keywords: shear bond strength, adhesive system, chlorhexidine gluconate 2%, matrix metalloproteinases, MMPs

Introduction

Dental and oral health problems are quite a big issue in Indonesia. The 2018 Basic Health Research (Riskesdas) data shows that damaged teeth, cavities, and toothache make up the largest proportion of dental problems in Indonesia (45.3%).[1] Dental restoration is a curative treatment that can repair damaged tooth structure to restore its form, function, and aesthetic.[2]

Composite resin is one of the materials that can be used to restore teeth. This material can be used in various types and sizes of cavities.[3] Composite restorations have several advantages such as requiring conservative preparation that results in minimal extension, low thermal conductivity, economical material, and ability to improve dental aesthetics.[4] However, if the composite restoration procedure is not carried out properly, it can cause secondary caries and fracture of the restoration.[5] Astvaldsdottir states that the most common reason for restoration failure is secondary caries and fractures.[6,7] In addition, restorations can also experience adhesive failure arising from a lack of retention of the composite against the tooth surface.[8] Costa has conducted a clinical evaluation of composite resin restorations on posterior teeth that concludes 57.5% of composite restorations failed, about 46.7% were caused by secondary caries and 19.3% were caused by fracture.[6]

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Restoration failure can be caused by both operator and material factors.[9] Differences in chemical formulation, physical properties, and composite insertion techniques can affect the durability of composite restorations.[10] One of the causes of composite restoration failure is the low resin bond to enamel or dentin.[9] The durability of composite restorations can be reduced due to the degradation of the hybrid layer, namely the layer between the adhesive resin and dentin collagen.[2,11] Khabadze stated that the enzyme activity of matrix metalloproteinases enzymes (MMPs) is responsible for the degradation of the hybrid layer.[12] In addition, Sullivan stated that MMPs have a major role in restoration failure.[13]

MMPs are enzymes present in the organic components of dentine.[14] In a mineralized state (healthy dentin), MMPs are inactive, and even so, MMPs can be activated by acids from cariogenic bacteria and/or acidic materials used in restorative procedures such as phosphoric acid and acid monomers.[14] MMPs that are already active can degrade and segment collagen in dentine into several parts which then cause a degradation effect on the hybrid layer.[15] Degradation of collagen fibers will cause the bond between collagen fibers and bonding material to decrease which can lead to reduction of the hybrid layer integrity that will reduce the bond strength of the bonding material to the dentine and result in failure of the composite restoration.[11,16,17]

The activity of MMPs caused by etching in restorative procedures can be reduced by applying MMPs inhibitors.[16] One of the ingredients that can act as an MMPs inhibitor is chlorhexidine, this material can reduce MMPs activity by a chelating mechanism.[14] Application of chlorhexidine can reduce MMPs activity and is beneficial in increasing the strength of the hybrid layer.[15]

A previous study which was carried out by Rayar stated that the application of 2% chlorhexidine gluconate for 60 seconds effectively increased the shear bond strength and helped maintain the durability of the bonding agent interface with the total-etch and self-etch systems.[18] Another study was conducted by Deniz which stated that the application of chlorhexidine for 30 seconds in the immediate dentin sealing procedure with a universal adhesive system could increase the shear bond strength of the resin cement to the dentin.[16] In addition, Puspitasari stated that there was an increase in the bond strength of the resin to dentin following the application of 2% chlorhexidine gluconate for 15 seconds compared to without application of 2% chlorhexidine gluconate.[19] However, the difference in bond strength was not statistically significant.[19] The above studies showed that there was an effect of 2% chlorhexidine on increasing the shear bond strength of the resin to dentin, even so, the effective duration of the application of 2% chlorhexidine is still not known with certainty.

The purpose of this study is to determine the role of the duration of chlorhexidine gluconate 2% application on shear bond strength of a total-etch bonding agent. The null hypothesis was as follows: H0: there were no significant differences in the shear bond strength of the dentin bonding agent after application of 2% chlorhexidine for different durations.

Materials and Methods

Preliminary Stage

The type of research used was laboratory experimental research conducted at the Integrated Research Laboratory of the Faculty of Dentistry, Universitas Padjadjaran, Indonesia. This research has received approval from the Universitas Padjadjaran Research Ethics Commission with No. 569/UN6.KEP/EC/2023 and approval from the Dean of the Faculty of Dentistry, Universitas Padjadjaran, Indonesia no. 2827/UN6.F.1/PT.01.04/2023. This study used 36 newly extracted maxillary premolars as samples (less than 6 months after extraction), according to ISO standard 29022. The sample size was determined in accordance with the Central Limit Theorem. The teeth were collected from patients who indicated a need for extraction due to orthodontic treatment and provided consent for their teeth to be used for research purposes.

All of the teeth were cleaned and stored in 0.04% thymol solution until the next procedure.[20] The teeth were embedded in acrylic resin in a 1.8 cm × 2 cm PVC pipe. The tooth was then cut on the occlusal part by one-third of the crown using a carborundum disc until the dentine was exposed and then smoothed using polishing discs until the surface was even. The teeth were randomly divided into four groups with each group consisting of nine premolars: in group A (control), chlorhexidine gluconate 2% was not applied; in group B, chlorhexidine gluconate 2% was applied for 30

Table 1 The Composition of the Materials Used in This study

Brand Names	Manufacturer	Composition
Scotchbond Etchant	3M ESPE (St. Paul, MN)	32 wt% phosphoric acid, 60% water, 5% synthetic amorphous silica
Single Bond Universal	3M ESPE (St. Paul, MN)	MDP phosphate monomer, HEMA, dimethacrylate resins, vitrebond copolymer, filler, ethanol, water, initiators, silane
Filtek™ Z250 XT	3M ESPE (St. Paul, MN)	BIS-GMA, UDMA, BIS-EMA, TEGDMA. Silica particle 20 nm and Zirconia/Silica particle 10–0.1 microns (%67.8 by volume)

Notes: Data from these studies.[16,22]

seconds; in group C, chlorhexidine gluconate 2% was applied for 60 seconds; and in group D, chlorhexidine gluconate 2% was applied for 90 seconds. This study aims to determine the effect of the application duration of 2% chlorhexidine gluconate on the shear bond strength of bonding agents with a total-etch system.

Treatment Stage

The dentine was etched for 5 seconds using 37% phosphoric acid (Scotchbond Etchant, 3M ESPE St. Paul, MN) to remove the smear layer and minimize the dentin collagen degradation, then rinsed and air-dried lightly until the dentine was visibly damp.[21] Chlorhexidine was applied to the dentin according to predetermined groups. The excess chlorhexidine was removed using blotting paper after the assigned application duration per group.[18] Sequentially, a universal adhesive (Single Bond Universal, 3M ESPE, St. Paul, MN) was applied using a micro brush to the entire specimen for 20 seconds, light air pressure was given for 5 seconds and light-cured for 10 seconds.[16] The composite (Filtek™ Z250 XT) was applied on a dentin surface using a cylindrical mold made of polypropylene with a height of 2mm and a diameter of 2.5mm (Table 1).[18] The composite was inserted into the mold and condensed using ash 49. The excess composite was cleaned; then, the remaining composite on the mold was light-cured for 20 seconds. All specimens (Figure 1) were immersed in artificial saliva, consisting of NaCl 12mM, KSCN 3.4 mM, NaHCO3 17.8 mM, KCl 16 mM, Urea 22 mM, Na2HPO4 1.5 mM, and KH2PO4 1.5 mM, in an incubator (Memmert, IN-30) at 37°C for 24 hours prior to testing.[16]

Testing Stage

The specimen was placed and fixed into the jig on the universal testing machine. The chisel edge was mounted perpendicular to the specimen (Figure 2), then pressure was applied to the specimen. The universal testing machine tool stopped automatically when a fracture occurred in the interface between composite restoration and dentin surface.

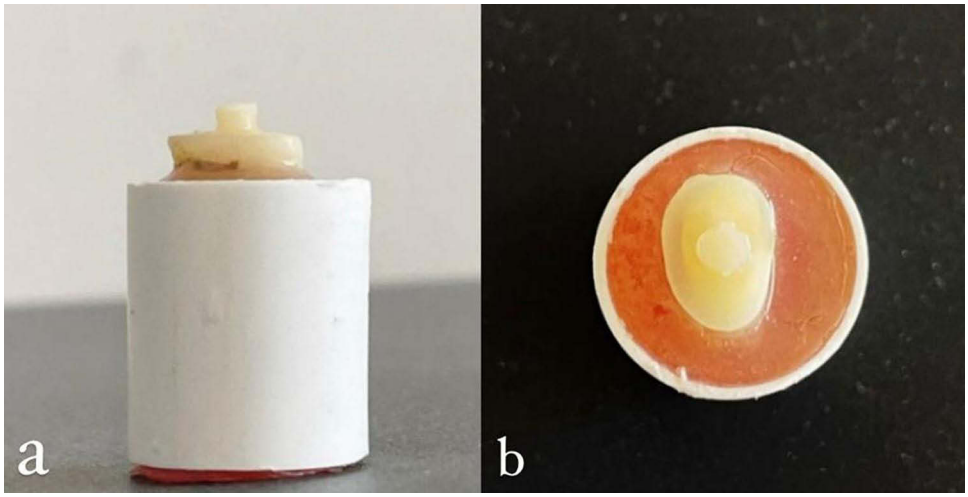

Figure 1 Specimen. (**a**) Specimen from lateral view, (**b**) specimen from occlusal view.

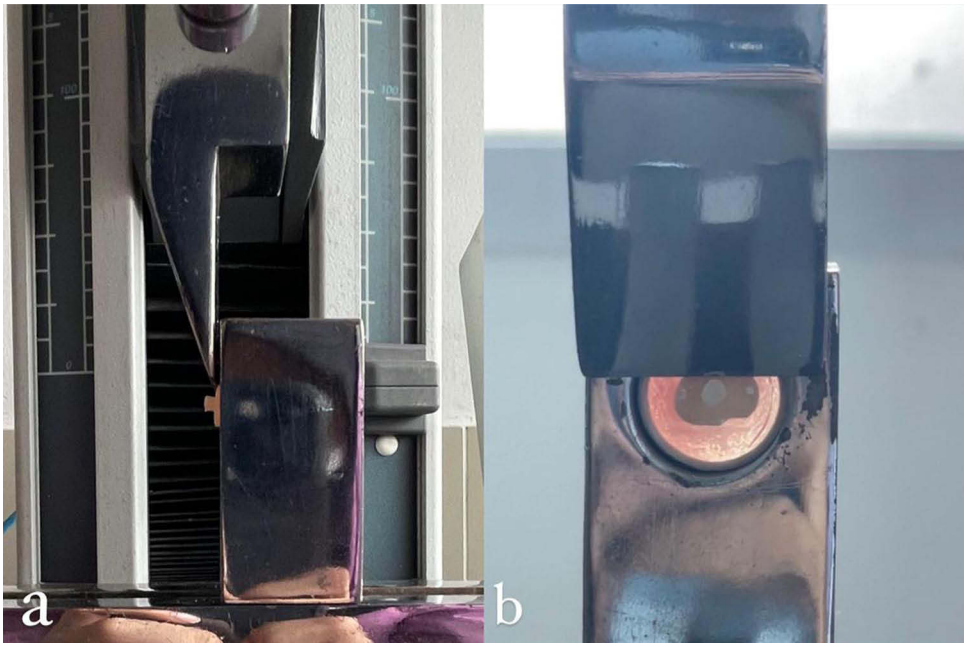

Figure 2 (**a**) Testing of composite shear bond strength using universal testing machine from lateral view, (**b**) testing of composite shear bond strength using universal testing machine from occlusal view.

The test results were recorded on the computer automatically, and then the shear bond strength of the composite was measured in MPa units using the formula S = F/A (S = Shear strength (MPa), F = Force (N) and A = Surface area (mm2)).[23] Furthermore, the specimens were observed using a macro lens camera (Nikon Z50 Macro Lens 85mm) to determine the type of failure that occurred.

Results

Shear Bond Strength

Table 2 shows the average value (mean) of shear bond strength and the standard deviation of all groups. The results of the one-way ANOVA statistical test are presented in Table 3. The table shows a significant p-value of 0.016 < 0.05; thus, H0 is rejected and H1 is accepted, which indicates that there is a significant difference in the shear strength of the dentine bonding agent after 2% chlorhexidine application with different durations.

In Table 4, the result of Tukey's test shows the average ratio of shear bond strength in each group. It can be concluded that the average value of shear bond strength in group A (control) is significantly different from the average value of shear bond strength in group C (60 seconds of chlorhexidine) and group D (90 seconds of chlorhexidine), while the average value of shear bond strength in group C (60 seconds of chlorhexidine) was not significantly different from the average value of group D (90 seconds of chlorhexidine).

Table 2 The Average Value of Shear Bond Strength Along with Standard Deviation

Average Value			
Groups	**Mean**	**N**	**Std. Deviation**
Control	12.6451	9	4.18326
30 Seconds	16.9376	9	4.33319
60 Seconds	18.2330	9	4.30783
90 Seconds	18.4758	9	3.38292
Total	16.5729	36	4.55963

Table 3 One-Way ANOVA Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	197.436	3	65.812	3.972	0.016
Within Groups	530.223	32	16.569		
Total	727.659	35			

Table 4 Tukey's Test

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	30 Seconds	−4.29248	1.91888	0.135	−9.4914	0.9065
	60 Seconds	−5.58788*	1.91888	0.031	−10.7868	−0.3889
	90 Seconds	−5.83066*	1.91888	0.023	−11.0296	−0.6317
30 Seconds	Control	4.29248	1.91888	0.135	−0.9065	9.4914
	60 Seconds	−1.29540	1.91888	0.906	−6.4943	3.9035
	90 Seconds	−1.53817	1.91888	0.853	−6.7371	3.6608
60 Seconds	Control	5.58788*	1.91888	0.031	0.3889	10.7868
	30 Seconds	1.29540	1.91888	0.906	−3.9035	6.4943
	90 Seconds	−0.24277	1.91888	0.999	−5.4417	4.9562
90 Seconds	Control	5.83066*	1.91888	0.023	0.6317	11.0296
	30 Seconds	1.53817	1.91888	0.853	−3.6608	6.7371
	60 Seconds	0.24277	1.91888	0.999	−4.9562	5.4417

Failure Types Analysis

Table 5 shows the distribution of failure types from each group which is classified based on Bracher's research (2017). The results of the Kruskal Wallis statistical test (Table 6) show a significant p-value of 0.749 > 0.05, thus it can be concluded that each group displays no significant difference in failure. In all specimens, there were only two types of failure, which are adhesive and Mixed 2 failures (Figure 3).

Table 5 Failure Types

Specimen	Failure				
	Cohesive 1	Mixed 1	Adhesive	Mixed 2	Cohesive 2
A	0	0	6	3	0
B	0	0	6	3	0
C	0	0	5	4	0
D	0	0	4	5	0

Notes: Score 1: Cohesive 1: cohesive failure in dentin; Score 2: Mixed 1: Combination of adhesive and cohesive failure in dentin and bonding agent; Score 3: Adhesive: Bonding agent adhesive failure, Score 4: Mixed 2: Combination of adhesive and cohesive failure in bonding agent and composite resin; Score 5: Cohesive 2: cohesive failure in composite resin.

Table 6 Kruskal–Wallis Test

	Failure Types
Kruskal–Wallis H	1.222
df	3
Asymp. Sig.	0.748

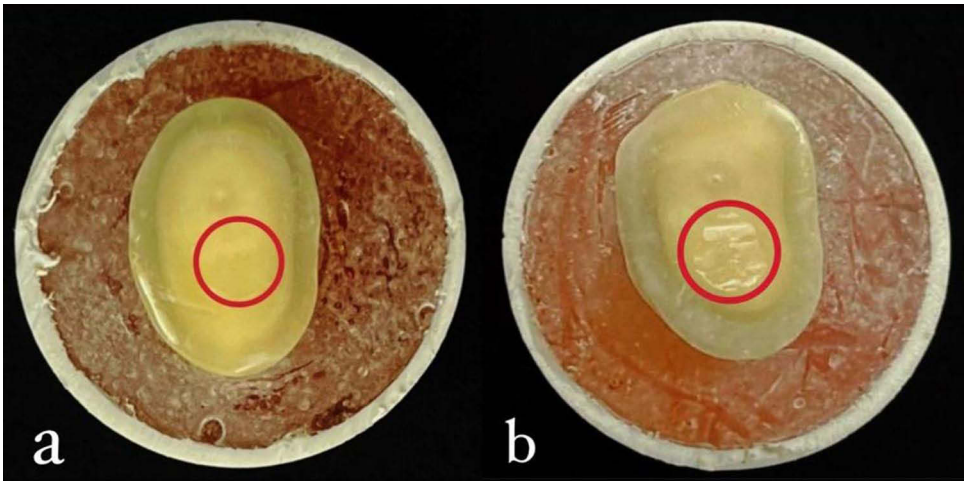

Figure 3 Failure types: (**a**) adhesive failure, (**b**) mixed 2 failure.

Discussion

Based on the results of this study, the average value of shear bond strength in the experimental group was higher than the average value of shear bond strength in the control group. The results of this study are parallel to the results of previous studies, which stated that there was an increase in the shear bond strength of the bonding agent with the total-etch system after the application of chlorhexidine gluconate 2%.[16,18,19,24,25] In this research, dentin was subjected to a 5-second etching process using a 37% phosphoric acid solution. This treatment effectively removed the smear layer, while also minimizing dentin collagen degradation. Furthermore, it successfully dissolved hydroxyapatite crystals present in both peritubular and intertubular dentin, resulting in the opening of dentinal tubules.[21] According to a prior study, it was found that the etching duration for dentin had no discernible impact on the clinical performance of adhesive restorations.[26]

Based on Table 2, these test values indicate an increase in the shear bond strength of the bonding agent along with the addition of the application duration of chlorhexidine gluconate 2%. This statement is in accordance with Puspitasari et al finding that differences in the duration of chlorhexidine application can affect the shear bond strength of composite resin to dentin.[19] Moraes et al stated that chlorhexidine can change the three-dimensional structure of MMPs enzymes and reduce the Ca^{2+} and Zn^{2+} ions that are needed to activate the enzymatic functions of MMPs.[11] Therefore, the longer the duration of chlorhexidine, the more Ca^{2+} and Zn^{2+} ions are chelated, resulting in more MMPs enzymes functions that are inhibited. However, this finding still needs further research.

In this study, an increase in shear bond strength began to be seen after 30 seconds, although this increase was not statistically significant. A study that was carried out by Ebrahimi et al stated that chlorhexidine can bind to collagen fibers in a short time, even in 30 seconds.[27]

Group C (60 seconds of chlorhexidine) showed a significant increase in shear bond strength compared to the control group. The value of shear bond strength in group C (60 seconds of chlorhexidine) was not significantly different when compared to group D (90 seconds of chlorhexidine), although the difference in duration was quite long. Therefore, based on this study, the duration of chlorhexidine gluconate 2% which is effective in increasing the shear strength of bonding agents with a total-etch system is 60 seconds. Rayar et al, in their study, stated that the application of chlorhexidine for 60 seconds effectively increased the shear bond strength with a total-etch system.[18] In addition, Ebrahimi et al stated that the application of chlorhexidine for 60 seconds was more effective than the application of chlorhexidine for 30 seconds in increasing the bond strength of the bonding agent to dentin.[27]

Restoration failure can occur cohesively, adhesively, or both. Bracher classifies the type of failure that consists of Cohesive 1 (cohesive failure in dentine), Mixed 1 (combination of adhesive and cohesive failures in dentin and bonding agent), Adhesive (adhesive failure of bonding agent), Mixed 2 (combination of adhesive and cohesive failures in bonding agent and composite resin), and Cohesive 2 (cohesive failure in composite resin). Based on the results of the failure analysis conducted in this study, it can be concluded that there is no significant difference in failure from each group. In all

specimens, there were only two types of failures: adhesive failure (adhesive failure of bonding agent) and Mixed 2 failure (combination of adhesive and cohesive failures in bonding agent and composite resin). Adhesive failure mostly occurred in group A (control) and group B (30 seconds of chlorhexidine), which was due to the poor bond between dentin and resin which was also indicated by the low value of shear bond strength in both groups.[16] Meanwhile, in group C (60 seconds of chlorhexidine) and group D (90 seconds of chlorhexidine), the most common failure was Mixed 2, which indicated a stronger bond than adhesive failure.[28] This matter was specified by a higher value of shear bond strength in group C and group D, compared to group A and group B. In this study, cohesive failure was not observed in any of the experimental groups. This phenomenon may be attributed to suboptimal material properties and bonding inadequacies.[8]

Observation of failure types in this study was carried out macroscopically through a macro lens. This method of observation is a limitation because the type of observed failure can be identified more accurately through microscopic observation of the hybrid layer and resin tag. Therefore, the use of scanning electron microscopy (SEM) is suggested to overcome the limitations that exist in this study.

Conclusion

From this study, it can be concluded that the application duration of chlorhexidine gluconate 2% affects the shear bond strength of the bonding agent with the total-etch system. The effective duration of chlorhexidine gluconate 2% for the restorative procedure is 60 seconds.

Disclosure

The authors report no conflicts of interest in this work.

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