

Comparing the Effect of two different deproteinizing agents on shear bond strength and resin dentin interface in dentin of primary teeth (In-vitro study)

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Aim: To compare the impacts of deproteinization with sodium hypochlorite versus chlorine dioxide on the shear bond strength & resin dentin interface of composite filling material to dentin in primary teeth.

Materials and methods: This investigation is an in-vitro investigation which included forty-eight human primary molars, extracted due to normal physiological resorption. Flat dentin surface was obtained (n=27) for evaluating shear bond strength, and 2 mm dentin slices (n=21) for evaluating resin dentin interface. Each deproteinizing agent has been applied to the dentin, followed by the usage of a universal adhesive in the self-etch mode. The control group didn't receive the deproteinizing agent. The shear bond strength of all samples has been assessed utilizing a universal testing machine. The failure mode has been measured by a stereomicroscope. The resin-dentin interface has been analyzed utilizing scanning electron microscopy.

Results: The highest bond strength has been found in chloride dioxide group, followed by the control group, while the lowest bond strength has been found in sodium hypochlorite group. Concerning mode of failure, sodium hypochlorite group having a significantly greater percentage of adhesive failures than other groups. Regarding resin tags length, the greatest resin tags length values were found in chloride dioxide, followed by the control group, while the lowest values was found in sodium hypochlorite.

Conclusion: Chlorine dioxide had the highest bond strength value while sodium hypochlorite showed lower value than control group. The highest adhesive failure was reported by the sodium hypochlorite while the higher mixed failure was reported for chlorine dioxide.

Keywords: Deproteinizing agents; resin dentin interface; shear bond strength; primary teeth

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Introduction

Composite restoration is widely popular in pediatric dentistry due of its pleasing aesthetic and conservative cavity preparation techniques. Hence, it is essential to undertake research for enhancing the strength and longevity of it's bond in primary teeth.¹

Dentin adhesion has shown to be more challenging and less reliable compared to enamel adhesion.

The challenge in bonding to dentin arises from the complex histologic structure and heterogeneous composition of dentin itself.² Moreover, in primary teeth the dentin has morphological characteristics, that can impact the effectiveness of adhesives. The primary tooth dentin has a lower mineral content comparison with permanent tooth dentin.²

The durability of dental restorations and their adhesive strength are determined by the degree to which polymerized resin penetrates into the demineralized dentin.³

The use of universal adhesive in self-etch mode may be favorable to overcoming the problems caused by acid etching and due to limited cooperation of children. However, these adhesives lack the ability to eliminate the smear layer, which consists of unorganized organic debris which binds mineral particles.⁴

The previous problem may be minimized or eliminated by using deproteinizing agent like sodium hypochlorite (NaOCl) and chlorine dioxide (ClO₂) to eliminate the smear layer & also produce a wettable surface like etched enamel, helping resin monomers to infiltrate through the demineralized interfibrillar spaces.⁵

Sodium hypochlorite is a chemical substance commonly used as a solution to clean & disinfect the root canal throughout endodontic procedures. NaOCl is the most frequently utilized deproteinizing agent to

remove excess protein. Nevertheless, there are conflicting reports according to the impact of NaOCl treatment on dentin bond strength. Some investigations have stated that NaOCl therapy enhances dentin bond strength, while others concluded that it has limited or no effect.⁶

To overcome some problems of sodium hypochlorite other deproteinizing agents such as chlorine dioxide (ClO₂).

Chlorine dioxide (ClO₂) has a disinfectant ability and employed in dental unit waterlines, certain commercial mouthrinses, and endodontic irrigants and additionally used for the sterilization of surgical and dental instruments⁷. ClO₂ chemically comparable to NaOCl, which has the ability to eliminate organic tissue, exhibits lower cytotoxicity compared to sodium hypochlorite NaOCl.⁸

Shear bond strength is the maximum stress a material can endure in a shear mode of loading prior to failure. Because it requires no additional specimen processing following the bonding procedure, the shear bond strength test is highly popular in research institutions. It is also the quickest and easiest method to use.⁹ While The purpose of scanning electron microscopy (SEM) evaluation of resin dentin interface is to detect the morphological properties of the resin-dentin interface and to learn more about their capacities for hybridization and penetration and quality of bonding.¹⁰

To the best of our knowledge limited investigation assessed the effect of ClO₂ on bond strength of primary teeth dentin for this reason the present investigation aimed to study the effect of ClO₂ as deproteinizing agent versus NaOCl on dentin of primary teeth.

The objective of this in vitro investigation was to compare the effects of deproteinization using sodium hypochlorite versus chlorine dioxide on the shear bond

strength & resin dentin interface of composite resin to dentin in primary teeth.

Materials and methods

This investigation is an in-vitro experimental investigation which included forty-eight human primary molars collected from patients after extraction due to normal physiological resorption. It was exempted from ethical review by Ain Shams University Research Ethics Committee (FDASU-REC) as it was an in vitro study that used teeth collected from anonymous patients.

Primary teeth with minimal or no caries were included in the study after extraction due root resorption and pre shedding mobility. While, primary teeth with Hypocalcification or white spot lesions, and teeth with structural defects, fracture lines or cracks have been excluded from the investigation.

Following extraction, all teeth have been cleaned using a hand-scaler and a mixture of pumice with a rubber cup connected to a low-speed contra-angle handpiece. This was done to eliminate dirt or particles on the surface of the teeth. The cleaned teeth were then preserved in a solution of 0.1 percent thymol at room temperature until they were ready to be used.

Sample size estimation:

A power analysis has been carried out to provide sufficient statistical power for testing the null hypothesis that there is insignificant variation in shear bond strength in the several groups being examined. By adopting the significance levels at $\alpha = 0.05$ and $\beta = 0.05$ (power = ninety-five percent) & considering an effect size of 0.998 as determined by Correr et al.¹¹, the estimated sample size (n) required for the study was 27. Concerning the resin dentin interface, we will use alpha and beta values of 0.05 (with a power of ninety-five percent) and an effect size of 1.03. The total number of samples in the investigation was 21, which

represents the sample size (n). The calculation of the sample size has been conducted utilizing G*Power version 3.1.9.

Forty-eight teeth were serially numbered & randomly divided 27 specimens for shear bond strength and 21 specimens for resin dentin interface evaluation each group was then randomly split into 3 subgroups regarding dentin pretreatment.

Shear bond strength test The teeth assigned for shear bond strength test have been embedded perpendicular to their long axis in acrylic resin (Acrostone™, Egypt) within polyvinyl rings with occlusal surfaces facing upwards after having their roots cut off 2mm below the cemento-enamel junction¹² Occlusal enamel was removed, utilizing a slow-speed diamond saw (Isomet 4000; Buehler, Lake Bluff, Ill., Gemany) under copious amount of coolant water. Specimens were then ground with 320, 400, & 600-grit silicon carbide paper¹³ (Leco; St. Joseph, Mich., USA) using a polishing machine (MetaServ 250 Twin; Buehler) under running water to create flat dentin surfaces with standardized smear layer¹⁴ Then teeth were randomly separated consistent with the dentin pretreatment used into: control group: no dentin pre-treatment and direct application of universal adhesive in self-etch mode according to manufacture instructions, NaCOI group 5.25%: sodium hypochlorite solution was applied for sixty second with disposable micro brush then rinsed with water for ten seconds, air-dried for five seconds. As for the chlorine dioxide group: A solution containing 0.12 percent chlorine dioxide has been applied for a duration of sixty seconds. Afterward, it has been rinsed with water for ten seconds & let too dry in the air for five seconds.¹⁵

Adhesive application: A dental adhesive (Single Bond, 3M ESPE, St. Paul, Minn., United States of America) has been applied to the dentin surfaces of all

specimens utilizing a disposable micro brush. The adhesive was applied for 20 second according to manufacturer instructions and then air-dried for five seconds. Afterward, an light emitting diode curing unit (Elipar Free Light II; 3M ESPE) with a light intensity of one thousand milliwatts per square centimetre has been utilized to light-cure the adhesive for ten seconds.¹²

Composite resin application:

A buildup was done using a custom-made Teflon mold (3 mm diameter x 4 mm height) to standardize the resin composite. Two layers of nanohybrid composite. (Filtek Z250; 3M ESPE) were applied using plastic instrument.¹⁶ The LED curing device has been utilized to polymerize each two-millimeters layer for a duration of twenty seconds. The specimens have been immersed in distilled water for preservation until they underwent the testing procedure.¹⁵

The tooth specimen, which had a bonded composite, was affixed to the lower fixed head of the universal testing machine (specifically, the Instron model 3345 from England). The experiment involved using a chisel with a blade width of half millimetre, which was put as close as possible to the interface between the composite and the slice. A compression force has been applied to the chisel blade in a controlled manner, using a crosshead speed of 1.0 millimetre per minute, until the specimen failed. The failure force (measured in Newtons) has been divided by the surface area (measured in square millimetres) to detect the shear bond strength in megapascals using the Blue Hill three Instron machine software from England.¹⁵

Assessment of mode of failure:

Following the shear bond test, all samples were examined under stereomicroscope at 40x using a Nikon SMZ745T Stereo microscope. The pictures were then taken & transferred to an International Business Machines personal

computer that had analysis software Buehler USA. The objective of this examination was to detect the failure mode pattern using the following categorization.¹⁵ Cohesive failure: refers to the fracture that happens within the dentin or resin composite. Adhesive failure: refers to the fracture at the contact among the adhesive & dentin. Mixed failure: is a combination of adhesive & cohesive failures, where the fracture occurs specifically at the interface among the adhesive or dentin, with part of the resin composite remaining on the dentin.

Evaluation of the Resin or Dentin Interface:

To assess the impact of prior treatment on the dentin resin interface, a total of twenty-one slabs, each measuring two millimeters in thickness, have been chosen. These slabs were divided equally among three groups, with seven slabs in each group. The therapy administered to the slabs was carried out following the same procedure as the SBS method.¹⁸ For Scanning electron microscope preparation, the specimens have been polished using wet silicon carbide with grit sizes of 600, 1200, and 4000.¹⁹ They were then cleaned using ultrasonicated in deionized water and ninety-five percent ethanol. After air-drying, the specimens have been mounted on aluminum tubs and examined using a scanning electron microscope to assess the morphology of the resin/dentin interfaces.²⁰ A sequence of photographs was captured, one by one, to observe the dentine/resin interface. The length of resin tags has been determined using a caliper, with reference to the scale provided on the photograph.²¹

Statistical analysis:

Fisher's exact test has been utilized to analyze categorical data, which have been presented as frequencies & percentages. Subsequently, pairwise comparisons have been carried out by multiple z-tests with Bonferroni correction. The mean and

standard deviation values have been utilized to present numerical data. The data distribution has been examined for normality & variance homogeneity via Shapiro-Wilk's & Levene's tests, respectively. They have been determined to be normally distributed with homogeneous variances across groups and have been subjected to one-way ANOVA followed by Tukey's post hoc test. Spearman's rank-order correlation coefficient has been utilized to conduct correlation analysis. In each test, the significance level has been established at p-value fewer than 0.05. R statistical analysis software version 4.4.0 for Windows has been utilized to conduct the statistical analysis.

Results

Intergroup comparison for shear bond strength (MPa) are exhibited in table (1). There was a significant distinction among distinct tested groups (p-value less than 0.001). The highest bond strength has been discovered in chloride dioxide group (25.95±4.34) (MPa), followed by the control group (19.12±4.04) (MPa), while the lowest bond strength has been found in NaOCl group (12.84±4.98) (MPa). All post hoc pairwise comparisons were statistically significant (p-value fewer than 0.001).

Table 1: Intergroup comparison and summary statistics for shear bond strength (MPa).

Shear bond strength (Mean±standard deviation) (MPa)			p-value
Control	Sodium hypochlorite	Chloride dioxide	
19.12±4.04 ^B	12.84±4.98 ^C	25.95±4.34 ^A	<0.001*

Values with different superscripts within the same horizontal row are significantly different *; significant (p<0.05).

Intergroup comparison and summary statistics for failure mode are presented in figure (1). There was a significant variance among tested groups, with sodium hypochlorite having a significantly greater percentage of adhesive failures than other groups (p-value equal 0.015).

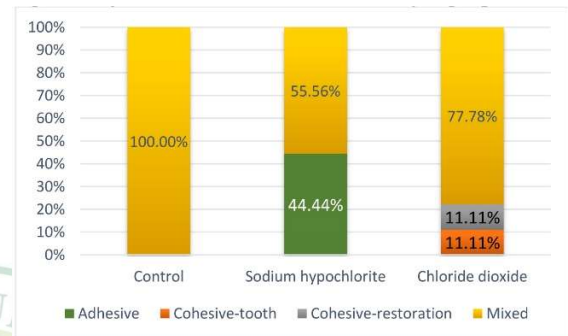


Figure 1: Stacked bar chart showing failure mode distribution.

Intergroup comparison for resin tags' length (µm) are exhibited in table (2) & figure (2). There was a significant distinction among distinct tested groups (p-value less than 0.001). The greatest length was found in chloride dioxide (52.80±4.92) (µm), followed by the control group (25.03±3.27) (µm), while the lowest value was found in sodium hypochlorite (12.24±2.49) (µm). All post hoc pairwise comparisons were statistically significant (p<0.001).

Table 2: Intergroup comparison and summary statistics for resin tags' length (µm).

Resin tags' length (Mean±SD) (µm)			
Control	Sodium hypochlorite	Chloride dioxide	p-value
25.03±3.27 ^B	12.24±2.49 ^C	52.80±4.92 ^A	<0.001*

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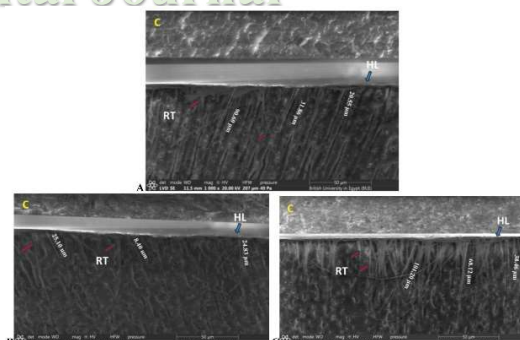


Figure 2: Representative scanning electron microscope images of the dentin resin interface of (A) control, (B) sodium hypochlorite (NaOCl) and (B) chlorine dioxide group (ClO₂) group, (C): resin composite.

Discussion

Composite restorations have gained a lot of attention in pediatric dentistry. In modern restorative dentistry, bonding restorative materials to enamel has become a regular and dependable procedure. Dentin exhibit complex histological features make bonding more challenging. The degree to which polymerized resin permeates the demineralized dentin determines the strength of the adhesive & the longevity of dental restorations. Due to children's limited participation, using universal adhesive in self-etch mode may be beneficial to save child cooperation. When it comes to enhance bond strength by eliminating excess protein in smear layer and replacing it by resin infiltration, NaOCl is the most commonly utilized deproteinizing agent. ClO₂ and other deproteinizing chemicals can be used to resolve some of the issues with sodium hypochlorite.^{4,6}

At present, there is insufficient evidence about the impact of chlorine dioxide as a deproteinizing agent on the dentin of primary teeth. The goal of our investigation was to assess & compare the impact of NaOCl with ClO₂ on the shear bond strength of dentin, as well as the mode of failure, and to examine the interface among the dentin & resin.

The primary mandibular second molars have been chosen because they had an appropriate mesio-distal width. Sound, rather than decayed, primary teeth were selected to assure a higher level of accuracy and reliability in the results.²²

The shear bond strength test (SBS) is most frequently chosen test to evaluate the bond strength. This test is widely used to assess the adhesive's bonding performance because it is quick, easy, and does not require any additional specimen preparation or specialized equipment.²³ Candan et al. (2023) concluded that shear testing is less technique sensitive as it does not need further

processing of specimens after the bonding procedure and more accurately matched clinical settings compared to tensile tests. Therefore, we employed the SBS test in our research.²⁴

The current research exhibited that there was a significant distinction among the different tested groups regarding shear bond strength. The greatest strength of bond has been discovered in chloride dioxide (25.95±4.34) (MPa), while the lowest bond strength has been found in sodium hypochlorite (12.84±4.98) (MPa) which was lower than control group.

This goes in harmony with previous Studies²⁵⁻²⁸ which claimed that the decrease in bond strength after dentin treatment with NaOCl can be attributed to the production of O₂ following the breakdown of NaOCl into NaCl & O₂.²⁷ The oxygen produced during this chemical reaction inhibits the polymerization of adhesive agents. In addition, the reactive remaining free radicals in sodium hypochlorite-treated dentin inhibit the propagation of vinyl free radicals produced throughout the light activation of the adhesive system. This results in premature chain termination & incomplete polymerization.^{25,26}

Regarding the influence of NaOCl application on SBS of the tested materials the majority of studies supported the present findings. Systematic review by Alshaikh et al. (2018)²⁹ and investigations by Delgado et al. (2022)³⁰ and Gonulol et al. (2023)³¹ stated that the bonding of self-etch adhesives to dentin is adversely affected by dentin surface pretreatment with NaOCl. This is due to the prolonged application of deproteinizing agents, which results in the destruction of more collagen scaffolds, resulting in a significant decrease in the number of binding sites for adhesive primers.

In addition, Taniguchi et al. (2019)³² concluded that the surface pH of dentin treated with NaOCl had significantly had

greater values compared to untreated dentin surfaces. The increased alkalinity of surfaces treated with NaOCl can be attributed to an elevated level of hydroxyl groups on the dentin surface. These hydroxyl groups act as buffers, neutralizing the acidity of self-etch adhesives. As a result, the capacity of the adhesives to form a strong bond with the underlying dentin is reduced.

Nevertheless, our outcome contradicted with the results of Elkassas et al. (2014),³³ Bahrololoomi et al. (2017)³⁴ and Haralur (2022)³⁵, who observed that the application of NaOCl before bonding enhanced the adhesive's bond strength. The conflicting outcomes attributed to different variations between studies such as permanent dentin instead of primary dentin as well as applying different NaCOI concentration with variable application time.

Conversely, the highest shear bond strength values have been reported in the ClO₂ group. This might be attributed to the limited tissue dissolving ability of ClO₂ and also on smear layer removal compared to the NaOCl. One possible explanation is that ClO₂, which is a weak acid, may create a mineral gradient that is more favorable to resin infiltration. Moreover, it was reported that using ClO₂ results in more resin infiltration in exposed collagen network which leads to higher bond strength values.³⁶ High ClO₂ values in our research came in accordance with Bedir & Telatar (2023)³⁷ who aimed to assess the impact of various dentin deproteinization agents on the bond strength of composite resin to the dentin of primary and permanent teeth dentin. They reported that the values for the ClO₂ groups were significantly greater than those of both the control & NaCOI groups. Although NaOCl is widely used for dentin deproteinization, a chlorine dioxide became more recommended as it results in improving dentin bond strength for both primary & permanent teeth.

In the current study, the fractured samples in each group were examined utilizing a stereomicroscope to identify the mode of failure. Which provides significant information for a more precise interpretation of bond strength values.³⁸

As regards to, the fracture mode analysis in the present study mixed failures were predominant in all groups. However, NaCOI group exhibited a higher percentage of adhesive failures compared to the rest of the groups. Adhesive failure indicated that the resin dentin bond was weakened while mixed mode of failure indicates higher bond strength.

This is consistent with Coelho et al. (2021),³⁹ who reported that a greater percentage of mixed failure are related to high bond strength in ClO₂ group while the adhesive fracture are associated with low bond strength in NaCOI group.

In the current research resin tags evaluation was advocated since, the length of resin tags is important for the bonding process among composite resin and dentin. This bonding depends on the hydrophilic monomer permeating into the dentin, followed by resin penetration into partially demineralized dentin. This process leads to the creation of resin tags and the establishment of a hybrid layer or resin-dentin interdiffusion zone⁴⁰. The resin tag length is believed to contribute most to the retention & sealing efficiency of most dental adhesives.⁴¹

Our result demonstrated that the highest mean value of resin tags length was found in ClO₂ group followed by the control group while the lowest mean value has been found in the NaCOI group. This may be attributed to the fact that chlorine dioxide removed a part of the remaining smear layer along with some of the residual material and created wide dentinal tubules with parallel direction and regular surface, allowing the adhesive to further penetrate in micro-tags.⁴²

The aforementioned results come in agreement with Coelho et al. (2021)³⁹ who stated that NaCOI displayed shallow resin tags and a generalized interfacial gap formation with no effect on smear layer and incomplete resin infiltration. Similarly, According to Alkhudairy et al. (2018),⁴³ who conducted a investigation on the impact of NaCOI on the bonding strength of cemented fiber posts & resin cement-tags in root canal dentin, they found that the group treated with sodium hypochlorite had lower creation of resin tags & inadequate removal of the smear layer.

On the other hand, Gowda et al. (2012),⁴⁴ found that SEM had shown remarkable alterations to the morphology of the demineralized primary dentin with longer resin tags and deeper resin penetration. Which provided either is by increasing the concentration to be 10% instead of 5.25% utilized in our study or by using long deproteinization time up to 2 mins instead of 1 min advocated in the present study.⁹ The present study has variable strength which include adopted rigorous measures to achieve maximum level of standardization like identical tooth preparation and application protocol for the used materials that was performed with a single investigator. Moreover, microscopic evaluation of mode of failure and resin dentin interface using SEM was performed by independent examiners who were calibrated and blinded. Statistician was also blinded about the groups and materials which in elimination of bias and increase study accuracy and reliability.

However, there are limitations that worth mentioning, for instance the in-vitro nature of the study does not typically mimic the oral environment. In addition, using small sample size in this study due to the difficulty to collect more teeth with inclusion criteria. Moreover, the chemical instability of the deproteinizing agents utilized which makes them difficult to store for a long time.

Conclusion

Regardless that sodium hypochlorite is widely used as deproteinizing agent Chlorine dioxide had the greatest bond strength value while sodium hypochlorite showed lower value than control group. The highest adhesive failure was reported by the sodium hypochlorite while the higher mixed failure was reported for ClO₂. Chlorine dioxide is better regarding shear bond strength, mode of failure & resin tags formation.

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Availability of data and materials

All data generated or analyzed during this study is included in this published article.

Declarations

Ethics approval and consent to participate

This study was exempted from ethical review by Ain Shams University Research Ethics Committee (FDASU-REC) as it was an in vitro study that used teeth collected from anonymous patients. The ethical approval number was (FDASU-RECEM012137) which was issued in January 2021.

Competing interests

None.

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