

The Effect of Sodium Hypochlorite Irrigation on Dentin's Collagen and Shear Bond Strength of Composite Resin to Dentin

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Abstract

Sodium hypochlorite (NaOCl) as a root canal irrigant during endodontic treatment, may dissolve organic part of the dentin structure, especially collagen. The aim of this study was to analyze the effect of irrigation with 2.5% and 5.25% NaOCl on collagen in dentin and shear bond strength (SBS) values of total-etch adhesive system to dentin with nanofilled composite resin NaOCl.

Fifteen human maxillary premolars were halved, which produced 30 specimens that were randomly divided into 3 groups of 10 each for Mallory Azan staining. For the SBS test, 10 different maxillary premolar were sliced into 3 parts each and were divided into 3 groups of 10 each. In each test, 2 of the groups were irrigated with 2.5% and 5.25% NaOCl, and one unirrigated group was used as control. Mallory Azan staining was performed to evaluate the presence of collagen. Evaluation of the specimens irrigated by different concentrations of NaOCl and control group revealed that increasing NaOCl concentration reduced the collagen in the treated dentin ($p < 0.05$).

It was shown that 5.25% NaOCl group had weak collagen staining but had the highest SBS value (15.56 ± 0.43 MPa), compared with 2.5% NaOCl group (9.2 ± 0.65 MPa) and control group (14.41 ± 0.96 MPa) ($p < 0.05$).

Thus, 5.25% NaOCl can be recommended as an irrigation in endodontic treatment because it increased the SBS.

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Introduction

Composite resin is an option for restoration of endodontically treated tooth. This adhesive restoration material bonds with dentin organic components through the formation of a hybrid layer by means of a resin monomer that infiltrates the collagen fibrils and hydroxyapatite.¹⁻⁴ Polymer-collagen binding results in the formation of a strong bond between the adhesive material and dentin, especially in the intertubular dentin.⁴ The bond can't be sustained easily because collagen fibril are prone to degradation by protease, and the resin is hydrolyzed by dentinal fluid.^{2,5,6} The collagen fibrils are also susceptible to

degradation by the chemical irrigation material in root canal treatment, such as: Sodium hypochlorite.⁷

Sodium hypochlorite (NaOCl) is a root canal irrigation solution used in endodontic treatment. The concentrations commonly employed are 2.5% and 5.25%. NaOCl exposure can dissolve up to 300 μm of an organic material, including collagen fibrils.⁷⁻¹³ The chemical induces loss of collagen fibrils through its deproteinizing action, leaving only the dentin mineral surface behind. This allows a direct adhesion between the adhesive resin and dentin without the formation of hybrid layer.¹⁴ Degradation of collagen fibrils is marked by the loss of carbon bonds in the collagen's primary structure.¹⁵ After fragmentation of the long collagen chains, NaOCl cleaves the protein's terminal groups into smaller units.¹⁴

This study aimed to analyze the effect of irrigation with 2.5% and 5.25% NaOCl on collagen in dentin and shear bond strength (SBS) values of total-etch adhesive system to dentin

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with nano-composite resin.

Materials and methods

Caries-free maxillary premolars extracted from humans aged 17–30 years were collected after obtaining the patient's informed consent under a protocol reviewed and approved by the Dental Research Ethics Committee, Faculty of Dentistry, Universitas Indonesia (No. 070230317). All extracted teeth were stored in phosphate-buffered saline (PBS) at -20°C until use.¹⁶ All the extracted teeth were stored in PBS to keep them as fresh as possible. The PBS solution had a pH of 7.2-7.4 and contained Na^+ , PO_4^{3-} , and Cl^- ions, which are necessary to maintain the electrolyte and pH balance for over a month of storage.¹⁷ The samples were kept at -20°C to preserve the morphology, and protein content of the dentin.^{16,18} The samples were not kept for more than 6 months after extraction according to the ISO technical specification 11405.¹⁹

Dentin collagen analysis

Fifteen teeth were longitudinally sliced bucco-palatally into 2 parts using a slow-speed saw (Ref. 58 0910D 104 220, Hager & Meisinger GmbH, Germany) under water coolant, produced 30 specimens that were randomly divided into 3 groups of 10 each ($n = 10$). Groups 1 and 2 were irrigated with 2.5% NaOCl (Onemed) and 5.25% NaOCl (Chloraxid; Cerkamed), respectively. The unirrigated group 3 served as control. The dentin surface in the medial part of the crown were irrigated with 45 mL of NaOCl solution for 12 min using an irrigation machine (Redent-Nova, Vatea) and a 30G needle to control the speed.⁹ The total volume was counted, starting from the beginning of root canal treatment up to the obturation moment, to simulate the clinical condition. Then, irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA; pH 7.0; endo-solution; Cerkamed) for 1 min,^{9,20,21} flushed with 5 mL of NaOCl for another 1 min, and finally dried with absorbent papers.⁹

All specimens were fixed with 4% paraformaldehyde (PFA) in PBS for 1 hour at room temperature and decalcified using 0.1 M EDTA for 3 weeks at 42°C .²² The specimens were x-rayed (SOPIX, Acteon, France) daily to monitor the progression of the demineralization process. Subsequently, they were embedded in paraffin and transversally cut bucco-palatally into

5- μm thick slices using a microtome. The sections were mounted on a glass slide, deparaffinized with xylene, hydrated in 100%, 96% and 80% alcohol sequentially in 3 min each,²³ and stained with Mallory Azan technique.²⁴ Scoring was performed based on the intensity of the stain. Intensity was categorized as nil, weak (light blue), moderate (yellow), or strong (dark blue) staining.²⁵ All slides were viewed under a stereo microscope (10x magnification; ZEISS Stemi 305, Germany). Each sample was examined and scored by an oral pathologist.

SBS test

Additional ten maxillary premolars were longitudinally cut bucco-palatally into 3 parts ($n = 30$), and the roots were removed. The slices were embedded in acrylic resin. The surfaces were ground semi automatically (LaboPol-21, Struers, Denmark) under constant water spray with 120-grit silicone to expose the flat dentin surface and were polished with 600-grit silicone carbide paper for 20 s to obtain a uniform smear layer.²⁶ Next, the samples were cleaned in aquabidest with ultrasonic cleanser for 5 min.

Specimens were then divided into 3 groups ($n = 10$). Groups 1 and 2 were irrigated with 2.5% NaOCl (Onemed) and 5.25% NaOCl (Chloraxid, Cerkamed), respectively. Group 3 was not irrigated and served as control. The volume and time of NaOCl exposure were the same as that for dentin collagen analysis.

Each specimen was etched using 37% phosphoric acid gel (ScotchbondTM, 3M ESPE) for 15 s, rinsed with water spray, and air dried for 5 s. The adhesive (AdperTM Single Bond 2, 3M ESPE) was applied on the dentin surfaces over 1-mm diameter using 0° angled microbrush tips by the scrubbing technique for 20 s and 3gr forces, followed by air thinning for 5 s. The adhesive was then light-cured for 10 s with an LED-curing unit (1100-1330 mW/cm^2) (DemiTM Ultra, Kerr, USA) at 2-mm distance.^{2,27} The acrylic with a cylinder template (1-mm diameter; 2-mm height) was placed on dentin's adhesive surface, then filled with nanofilled composite resin (FiltekTM Z350XT, 3M ESPE), and light-cured for 10 s.^{22,24-26}

Prior to testing, the diameter of each bonded resin cylinder was measured with an electronic digital caliper (Guanglu, 1-29, China) to confirm the bonding area. The assemblies were kept at 37°C for 24 h with 100% humidity.

Later, they were employed for the SBS test using a universal testing machine (AG-5000E, Shimadzu, Japan) at a crosshead speed of 0.5 mm/min and a load cell of 50 kgF until failure occurred.

Mode of failure after SBS tests

After the SBS tests, the de-bonded specimens were observed under a stereo microscope at 40x magnification (ZEISS Stemi 305, Germany) to determine the failure modes, which were divided into adhesive, cohesive, and mixed failures.²⁶

Statistical analysis

The differences in the collagen staining of the various groups were compared non-parametrically by Kruskal–Wallis and Mann–Whitney post-hoc tests. The normality of the data distribution was analyzed with Saphiro-Wilk. One-way ANOVA and Bonferroni post-hoc tests were utilized for comparing the SBS values. The data were analyzed using the IBM SPSS 20 software (SPSS Inc., Chicago, IL, USA), and the significance was set at 0.05.

Results

With Mallory Azan staining, the presence of collagen was indicated by a blue color. The significance of the collagen staining between the tested groups is presented in Table 1. Kruskal–Wallis test revealed significant differences among the groups ($p < 0.01$). Mann–Whitney test asserted that a significant difference existed between the 2.5% and 5.25% NaOCl groups. The 5.25% group showed the weakest collagen staining, while the control group showed the strongest collagen staining, compared with both NaOCl groups (Fig. 1). The mean SBS values (MPa), standard deviations, and statistical results of the materials are furnished in Table 2. One-way ANOVA revealed that the SBS of the composite resin to the dentin was significantly influenced by NaOCl ($p < 0.05$). The values for the 5.25% NaOCl group (15.56 ± 0.43 MPa) were significantly greater than those for the 2.5% NaOCl (9.2 ± 0.65 MPa) and control groups (14.41 ± 0.96 MPa). Bonferroni post-hoc test revealed significant differences in the SBS values of the 3 groups (Table 3).

	5.25% NaOCl	Control
2.5% NaOCl	0.039*	<0.001*
5.25% NaOCl	-	<0.001*

Table 1. Significance of collagen density between the tested groups.

Groups	n	Mean ± SD	P
2.5% NaOCl	10	9.2 ± 0.65	<0.001*
5.25% NaOCl	10	15.56 ± 0.43	
Control	10	14.41 ± 0.96	

*One-way ANOVA test with significance set at $p < 0.05$

Table 2. Comparison among the shear bond strength (Mpa) of the tested groups.

	5.25% NaOCl	Control
2.5% NaOCl	<0.001*	<0.001*
2.5% NaOCl	-	0.004*

*Bonferroni post-hoc test with significance set at $p < 0.05$

Table 3. Significance of shear bond strength among the tested groups

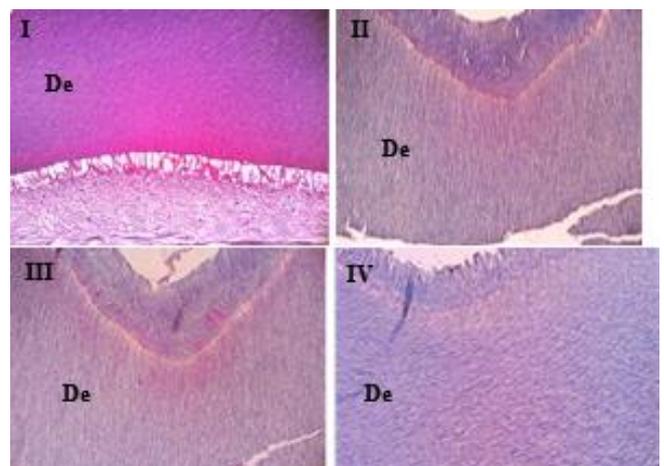


Figure 1. Mallory Azan histochemical staining for collagen in the coronal part of dentin (De). All slides were viewed under a stereo microscope (10x magnification; ZEISS Stemi 305, Germany) and scored with the following categories: (I) Strong; (II) Moderate; (III) Weak; (IV) No Staining.

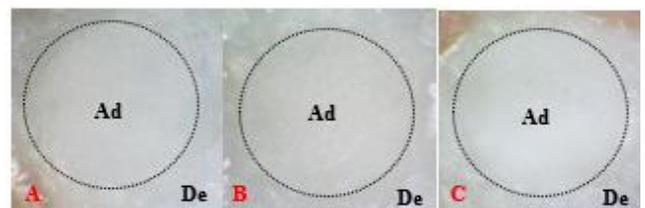


Figure 2. Observation of the failure modes of the shear test (40x). De: Dentin, Ad: Adhesive. A. Control group. B. 2.5% NaOCl group. C. 5.25% NaOCl group.

The samples could be categorized as adhesive failures. Adhesive failure occurred at the interface between the adhesive and the resin cylinder.

The failure modes of the SBS are depicted in Figure 2. Stereo microscopic observation ascertained that all the samples could be categorized as adhesive failures, in which the composite resin adhered to <25% of the total interfacial area.

Discussion

Dentin collagen plays an important role in the effective bonding procedure.²⁸ The reduction of the bond strength between adhesive systems and dentin walls might occur because of the removal of collagen fibrils from the dentin surface by NaOCl and might impede the formation of a consistent hybrid layer,²⁹ as the retention of the adhesive is primarily due to collagen.^{1,3,4} One of the factors related to bonding failures in the root canal system is the dentin collagen integrity, especially after treatment with endodontic irrigants.²⁹ This study proved that irrigation with 2.5% and 5.25% NaOCl reduced the collagen from dentin (Table 1). Dentin collagen alteration after treatment with chemical substances can be observed by Mallory Azan staining that the 5.25% NaOCl group had the weakest collagen density (Fig. 1). The higher the concentration, the more pronounced was the collagen degradation. This findings were in accordance with previous work that the increase in the concentration of NaOCl solution lead to an increase in the tissue dissolution and dentin collagen deproteination.^{30,31}

This study also analyzed the SBS of composite resin toward dentin after NaOCl exposure, because teeth can be restored with composite resin after an endodontic treatment, both directly and indirectly.³²

This research exposed that collagen may not be the primary retention factor for bonding. We found that the 5.25% NaOCl group had the weakest collagen staining, but the highest SBS value (Tables 1 and 2). The retention may be attributed to the penetration of the resin monomer by the inorganic portions of the dentin directly in the absence of collagen.¹⁴ These strong bonds can be achieved with a high concentration of NaOCl. Irrigation with 5.25% NaOCl removes the organic parts of the dentin,

especially collagen, effectively.

In this investigation, there was a discrepancy between the 2.5% and 5.25% NaOCl groups. It was discerned that the 2.5% NaOCl solution lowered the SBS value (Table 2). This effect may be attributed to the differing integrity of the collagen fibril that is neither totally lost nor completely intact, such that the hybrid layer is not optimally formed.³³ This result differs from those of previous studies which revealed that 5.25% NaOCl exerts a negative effect, thereby lowering the SBS.^{33,34} This contradicted result could have been due to the difference between the volume and exposure time of NaOCl. The volume and duration of irrigation in this study were based on the calculation assumption during root canal treatment because there is no consensus regarding the optimal volume and time of exposure in the irrigation activation method.³⁵ Otherwise, in our study, the negative effect of NaOCl was witnessed at a concentration of 2.5%. According to the result of this study, we do not recommend using 2.5% NaOCl because it lowered the SBS value below the control group (unirrigated group).

During the total-etch adhesive procedure, the clinicians must take care with the drying procedure after etching. In vital teeth, the dentin must be kept moist with gentle air drying with spray to prevent the collagen's collapse and increase the bond strength.^{33,36} Related to the result of this study, in the absence of pulp tissue and the decreased presence of collagen, we suggest to overdry the dentin surface after rinsing the etch before adhesive procedure, as the collagen wasn't the primary retention adhesive any longer.

In this study, 17% EDTA solution was used because it is often used with NaOCl as a chelating agent to get rid of the smear layer. Previous study observed that 5.25% NaOCl, whether or not associated with 17% EDTA, does not cause significant alteration in the dentin collagen.^{29,37} It means that the EDTA solution only alters the inorganic components of dentin.³⁸

The Azan-Mallory stain is one of commonly used techniques, wherein ≥ 3 dyes are combined. These multiple-dye stains offer the advantage of revealing a large number of tissue structures and marking different tissues histologically.²⁵ The stain combines aniline blue, orange G (stains proteins), and acid fuchsin (stains DNA and RNA). Collagen-containing

connective tissue appears as blue; the erythrocytes are orange-colored; while the chromatin, nucleoli, basophilic cytoplasm, and muscle cell cytoplasm are stained red. In the azocarmine and aniline blue (Azan) stain, a combination of the basophilic dye (azocarmine) with aniline blue results in the staining of the nuclei and basic structures in red. Conversely, collagen, mucus, and cartilage matrix are stained blue. The Azan-Mallory method is still used for detecting several tissue components present in various organs.

However, in the present study, NaOCl was used prior to the application of adhesive resins. Furthermore, the irrigation time of NaOCl, the cutting direction of the dentin, the bonding location in dentin, and the ages of the subject have to be considered as another reason for different results with another study. The presence of the collagen according to the tooth's cutting direction of this study should be evaluated by scanning electron microscopy for future studies. Aside from that, more studies are still needed to investigate further the correlation between NaOCl and the other parts of dentin, e.g. non-collagenous proteins, related to the composite-dentin interface.

Conclusions

NaOCl reduced the presence of collagen in the dentin; however, its effects on the SBS values varied with the concentrations. It was inferred that irrigation with 2.5% NaOCl resulted in the lowest SBS value, while that with 5.25% NaOCl gave the highest value. As the 5.25% NaOCl had the highest SBS value, we suggest that 5.25% NaOCl be used in general as a primary root canal irrigant in clinical endodontic procedure. The findings of this study can serve as a reference for composite resin restoration of the endodontically treated tooth.

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Declaration of Interest

The authors report no conflict of interest

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