Effect of Dentin Pre-treatment on Smear Layer Thickness. An Ultrastructural Study

Mohd Haidil Akmal Mahdan[1]*, Nur Sarahniza Samani[1], Syakirah Mohd Ridzuan[1], Mohd Maizam Maideen[2]

[1]Fundamental Dental and Medical Sciences Department, Kulliyyah of Dentistry, International Islamic University Malaysia, 25200 Kuantan,Malaysia.
[2]Optics Unit, Kulliyyah of Medicine, International Islamic University Malaysia, 25200 Kuantan, Malaysia

Abstract

The aim of this study was to investigate the morphological changes of etched dentin by sodium hypochlorite (NaOCl) pre-treatment method applied for different durations and smear layer (SL) thickness. Sixty human molars were used in this study and sectioned at the coronal part to expose a flat dentin disc. They were ground with #180- or #600-grit silicon carbide papers, to produce thick and thin SL respectively. All specimens were assigned into 6 groups; G1: control; G2: Acid Etch (AE); G3, G4, G5 and G6: AE + 10% NaOCl for 15, 30, 60, and 120s, respectively. Specimens were observed under a Scanning Electron Microscope and the photomicrographs were classified according to the following scores: 0: presence of SL; 1: No smear layer + non-altered collagen fibrils; 2: No smear layer + slightly altered collagen fibrils; 3: No smear layer + severely altered collagen fibrils; and 4: No smear layer and absence of collagen fibrils. Kruskal Wallis showed all groups treated with NaOCl solution were significantly different, and alteration regarding the collagen fibrils network. The higher the duration of NaOCl pre-treatment, the more alteration of collagen fibrils occured.

Keywords: sodium hypochlorite (NaOCl), smear layer (SL), collagen fibrils.

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*Corresponding author: Mohd Haidil Akmal Mahdan; e-mail: haidil@iium.edu.my

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Introduction

The acid-etch technique relies on micromechanical adaptation created by acid etching of dentin substrate followed by penetration of polymerizable monomers into the interprismatic spaces to form micro and macro resin tag [1,2],thus exposing the collagen fibrils network. Van Merbeek et al [3] found that bonding to dentin depends mostly on micromechanical retention promoted by resin infiltration in partially demineralized dentin with consequent hybrid layer tags formation. Nakabayashi et al [4] 1992 reported that there were some limitations of total etch technique which is its potential for incomplete penetration of demineralized dentin by adhesive resin. Furthermore, clinically, acid etching is one technique which applied to remove the smear layer, which consists of debris, saliva, and variety of oral microbes. This smear layer has been reported to have different characteristics mainly depend on the types of burs used. [5-6].

The formation of smear layer is often simulated by polishing the tooth surface with different grit silicon carbide papers. Reports had mentioned that smear layers can vary in their thickness, roughness and density, depending upon roughness level of abrasive papers and burs used [7,8]. It had been reported that thin dentin smear layers, which are created by fine abraded-paper, appeared as thin but compact, while those created by coarse abraded-paper appeared rougher and thicker, and these characteristics seemed to reduce the dentinal perfusion [9, 10] which may affect bonding. In addition, Tay et al observed that thicker smear layers somehow have no effect in bonding of composite resin to dentin [11].

Dentin consists of simplified mineral and several organic components. Sodium hypochlorite (NaOCl) that is used as pre-treatment in the research is well known to be as nonspecific proteolytic agent capable to remove organic materials. Sodium hypochlorite solution (NaOCl) is widely used in various dental procedures such as in endodontic preparation and chemical removal of carious lesion in dentin and it has been proven as a preferable deproteinizing and disinfecting action [12]. Sakae et al. [13] observed changes in bovine dentin upon NaOCl treatment,and concluded that the solution has the ability to remove some unwanted organic substances from dentin substrate. In addition, NaOCl solution had been reported to be effective in collagen removal and thus providing an optimum contact between adhesive resin and dentin substrate [14, 15]. The micro morphological structures in permanent tooth varied, thus their bonding behavior might differ. The application of NaOCl solution to that substrate would produce different alterations as this can be observed by focusing on the collagen fibrils pattern.

Therefore, the purpose of this study was to investigate the alterations of etched dentine by sodium hypochlorite pre-treatment method applied for different durations and smear layer thickness. The hypothesis tested was that nor the smear layer thickness and NaOCl pretreatment method would affect the alteration of collagen fibrils.

Materials and Methods

A 37% phosphoric acid gel (AlphaDent) and 10% sodium hypochlorite solution (NaOCl), were used in this study for demineralization and deproteinization protocols, respectively.

Experimental design and specimen preparation

Sixty sound, permanent and non-carious human molars stored frozen in 10% formalin solution were used in this study according to protocol approved by IIUM Research Ethic Committee (No.546).

The procedure is summarized in Fig.1. The teeth were sectioned manually into a flat disc each by using low speed cutting machine under running water. The occlusal third and apical root section were removed and the remaining were horizontally sectioned in a bucco- lingual direction at the area of mid coronal to produce a 3 mm thick dentin disc. Sixty discs were then produced and ground to wet #600 or #180 silicon carbide (SiC) and assigned into two main groups (thin and thick smear layer groups) *n=30*. The samples were then further divided into six sub groups which comprised of five discs per group.

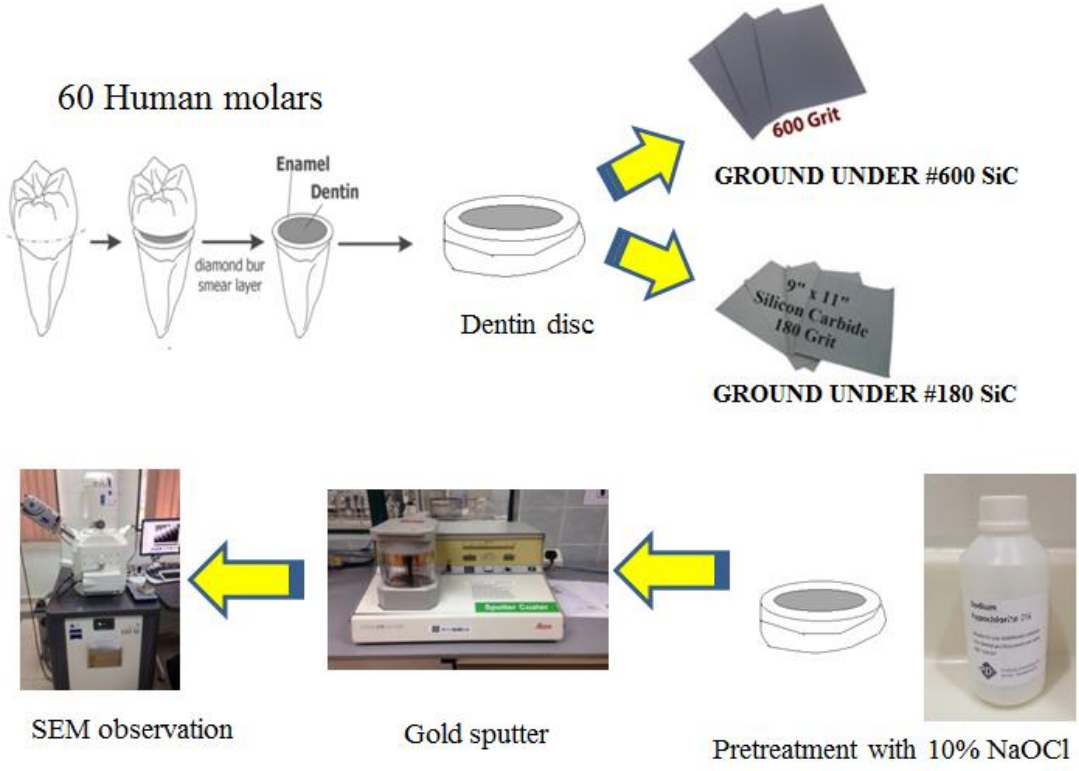


Figure 1: Schematic workflow

Group 1 served as control group (dentin surfaces rinsed with water only). Then, 37% phosphoric acid gel was applied to all the remaining 25 dentine discs and copiously rinsed for 15 seconds. After the demineralization protocol, five of the specimens were assigned into the Group 2. The dentin surfaces in Group 3were treated with 10% NaOCl for 15 seconds followed by water rinsing for 10 seconds. For the preparation of samples in Group 4, 5 and 6, the dentin surfaces were treated with 10% NaOCl for 30 seconds, 60 seconds and 120 seconds respectively and later rinsed with water for 10 seconds. The grouping of samples is shown in Table 1.

Table 1: Distribution of the experimental groups.

GROUP	DESCRIPTION
1	**rinse with water 10 sec ----CONTROL GROUP**
2	**AE + rinse with water for 15 sec**
3	**AE + treat with 10% NaOCl for 15 secs + rinse with water 10 sec**
4	**AE + treat with 10% NaOCl for 30 secs + rinse with water 10 sec**
5	**AE + treat with 10% NaOCl for 60 secs + rinse with water 10 sec**
6	**AE + treat with 10% NaOCl for 120 secs + rinse with water 10 sec**

After the pre-treatment with or without sodium hypochlorite solution, the dentin surfaces were dehydrated in different concentrations of alcohol as follows: 50% for 20 minutes, 75% for 20 minutes, and 100% for 60 minutes. These specimens were then stored be inside a covered glass vial at room temperature for 24 hours.

SEM observation

For observation under Scanning Electron Microscope (SEM, ZEISS Model SEM EVO50), the specimens were mounted on brass stubs and sputter-coated with gold using Sputter Coater machine (LEICA EM SCD005, Germany). After that, the discs were dried in vacuum and examined under SEM at a final magnifications at 2000x. Three images were taken in from one disc sample (15 images per group) the scoring of photomicrographs were based on the presence or absence of smear layer (SL) and the classic characteristics of collagen network. The scoring was listed in Table 2. The scores were then recorded and analyzed using Kruskal Wallis, followed by pairwise comparison test, using SPSS software version 16 at a significant level of $p < 0.05$.

Table 2: SEM analysis score

SCORE	ANALYSIS
0	PRESENCE of smear layer
1	**Absence** of smear layer and collagen fibrils remain intact.
2	**Absence** of smear layer and slight changes of collagen fibrils (less than 1/3rd of Collagen fibrils **were** removed)
3	**Absence** of smear layer, and severely altered collagen fibrils (More than 1/3rd of collagen fibrils **were** removed, few numbers of dentin porosities present)
4	**Absence** of both smear layer and collagen fibrils (higher number of intertubular porosities)

Results

The frequency of scores is shown in Figure 2. Kruskall Wallis analysis showed significant differences among the groups. In each group, pairwise comparison showed the thin smear layer preparation revealed a significant increase of SEM scores when compared to thick smear layer counterpart ($p<0.05$). For Group 3, most samples showed least occurrence of collagen alterations. In this group, only superficial collagen fibrils were removed by pre-treatment solution. When compared with the acid etched surface .In Group 4, thin smear layer showed more favorable reaction toward the NaOCl pretreatment compared to thicker smear layer,as more areas of collagen fibrils were being removed. There was a significant increase of specimens score with complete removal of collagen fibrils in thin layer preparation when compared to thick smear layer preparation in both Group 5 and 6. ($p<0.05$).

Figure 3 shows the images from Group 1 to Group 6 after ground with #600 Silicon Carbide paper. All images were observed under 2000x magnification of Scanning Electron Microscope. Group 1 acts as a control group (ground dentin). Dentin surfaces appeared to be compact and homogenous. After application of 37% acid etching in Group 2, the smear layer was removed and exposure of dentinal tubules (white arrow) can be seen in the image. After the application of NaOCl in Group 3,dentin surfaces showed presence of altered collagen fibrils. 4 ,5 and 6, there were alterations of collagen fibrils and exposed of secondary tubules in inter and

peritubular dentin (arrows in Group 6). As the duration of application NaOCl increased, more altered collagen fibrils and present of multiple iregularities of inter and peritubular dentin had been observed.

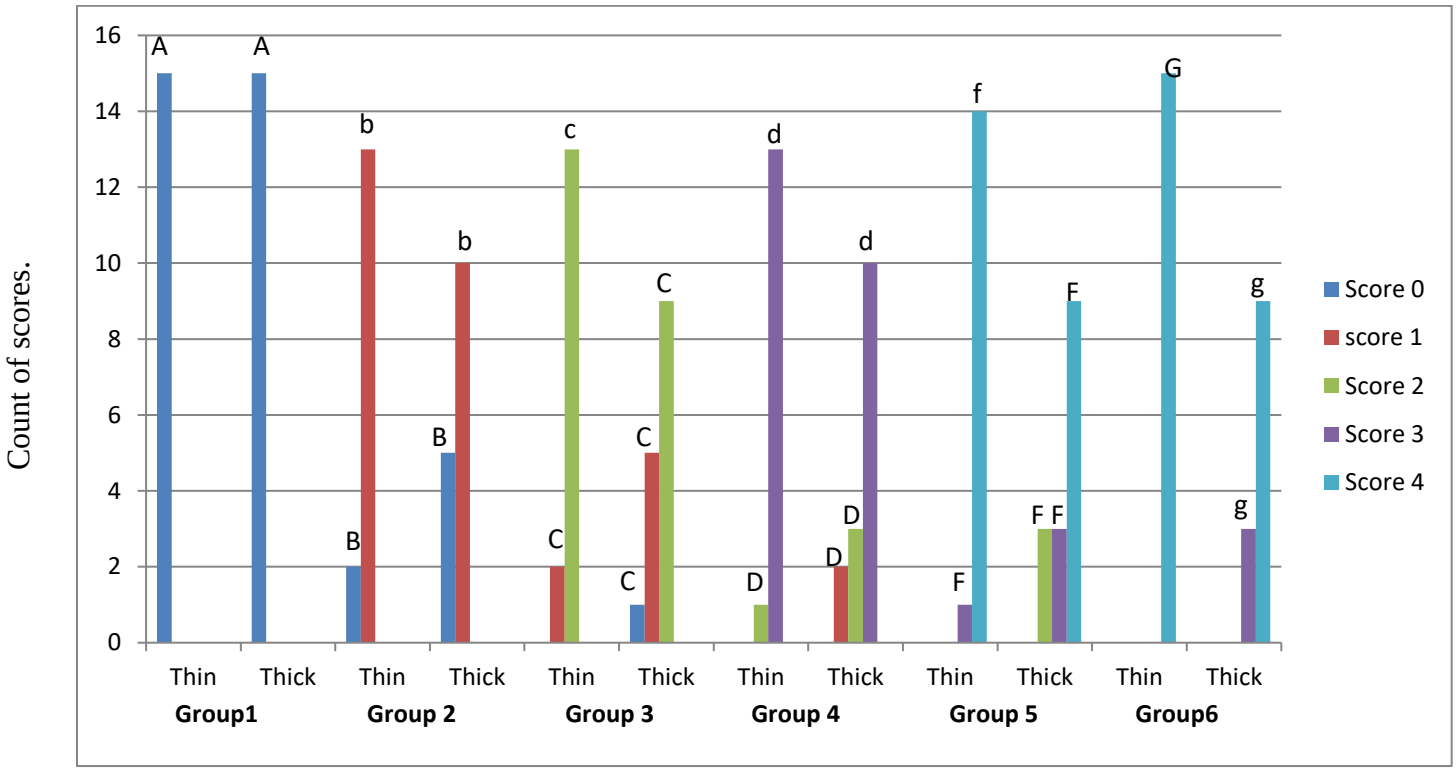
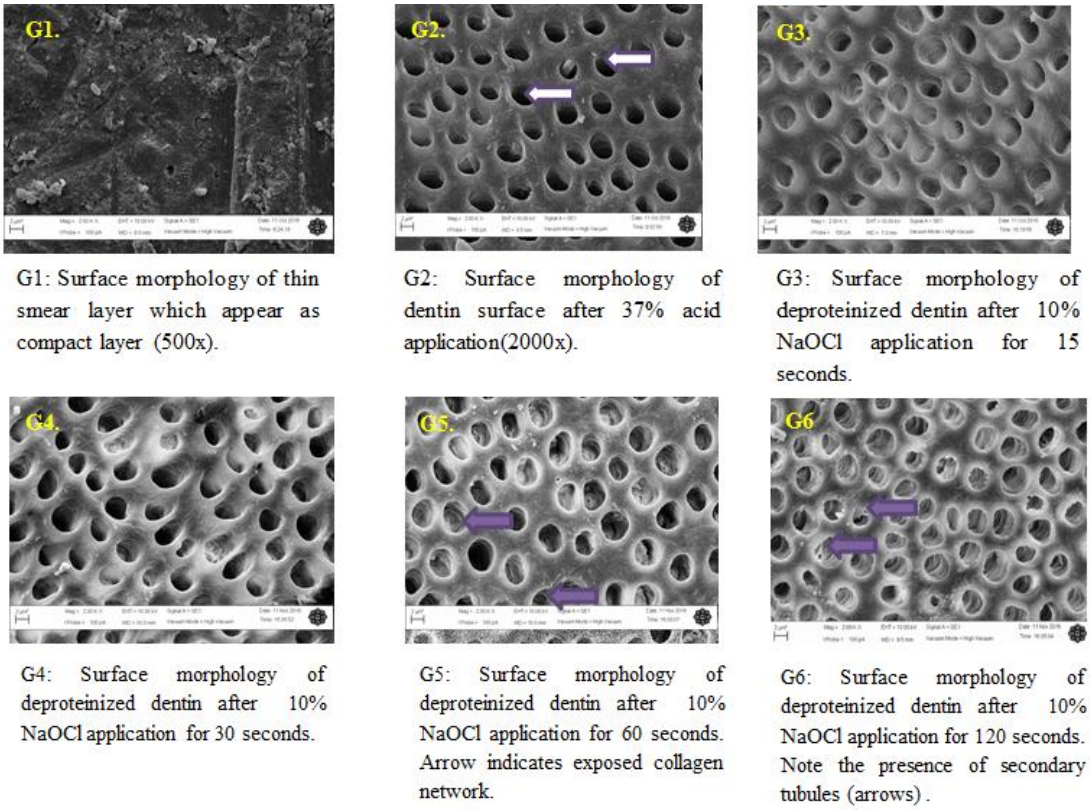


Figure 2: Scores frequency in the tested groups. Different uppercase and lower case letters within each group indicate statistical significant differences according to pairwise multiple comparison test ($p < 0.05$).



G1: Surface morphology of thin smear layer which appear as compact layer (500x).

G2: Surface morphology of dentin surface after 37% acid application(2000x).

G3: Surface morphology of deproteinized dentin after 10% NaOCl application for 15 seconds.

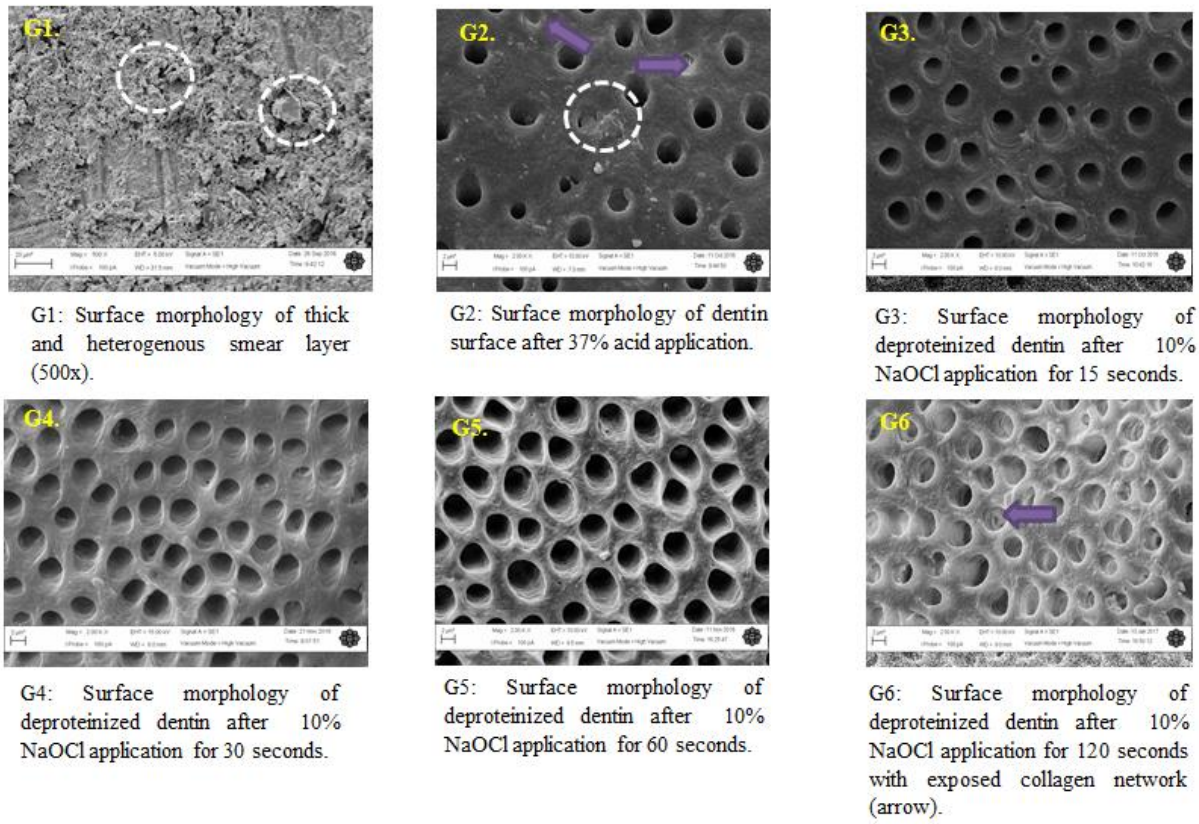
G4: Surface morphology of deproteinized dentin after 10% NaOCl application for 30 seconds.

G5: Surface morphology of deproteinized dentin after 10% NaOCl application for 60 seconds. Arrow indicates exposed collagen network.

G6: Surface morphology of deproteinized dentin after 10% NaOCl application for 120 seconds. Note the presence of secondary tubules (arrows).

Figure 3: Representative SEM images of dentin surfaces prepared in thin smear layer

Figure 4 below shows the images (x2000) from Group 1 to Group 6 after ground with #180 Silicon Carbide paper . G1 showed a thick smear layer and heterogenous in characteristic which is represented by remnants of smear debris covering the most areas of dentin surface(circle) produced by grinding with #180-SiC paper. After application of 37% acid etching in Group 2, presence of numerous smear plugs was observed (white arrows). In Group 3 to Group 6,similar patterns of surface characteristics was observed as there were lower evidence of secondary tubules exposure and lesser alterations of collagen fibrils compared to those in thin smear layer preparation.



G1: Surface morphology of thick and heterogenous smear layer (500x).

G2: Surface morphology of dentin surface after 37% acid application.

G3: Surface morphology of deproteinized dentin after 10% NaOCl application for 15 seconds.

G4: Surface morphology of deproteinized dentin after 10% NaOCl application for 30 seconds.

G5: Surface morphology of deproteinized dentin after 10% NaOCl application for 60 seconds.

G6: Surface morphology of deproteinized dentin after 10% NaOCl application for 120 seconds with exposed collagen network (arrow).

Figure 4: Representative SEM images of dentin surfaces prepared in thick smear layer

Discussion

In this study, the removal of smear layer was done using the action of acid etchant and sodium hypochlorite solution as this will help in further retention process of the adhesive and composite restoration respectively. Sodium hypochlorite pre-treatment, which usually done after acid etching, has been used in an attempt to increase the stability of resin-dentin interface. This technique is one way to minimize the hybridization technique sensitivity, producing a more durable adhesion to dentin substrate through its hydroxyapatite component [16].

Sodium hypochlorite solution causes an increase in physical properties of the dentin substrate. However, unremoved collagen fibrils could contribute to a damaging effect of the adaptation between resin and dentin interface. The results revealed that application of acid etch (Group 2) caused demineralization of the dentin with exposure of dentinal tubules. Dentin pre-treatment occurred from Group 3 to Group 6 by sodium hypochlorite which had formed a more porous structure of dentin substrate associated with the presence of numerous intertubular and peritubular porosities.

The alterations of the collagen fibrils were significantly observed with the increase in the duration of the sodium hypochlorite pre-treatment, when compared with the control group in Group 1 (ground dentin) and in Group 2 (demineralized dentin). In this study, it could be observed that as the time of application increased, there were also an increase in the quantity of the removed collagen fibrils as well as presence of a greater number of secondary tubules. In 120 seconds application, complete removal of collagen fibrils was detected regardless of smear layer preparation. This results had been supported by the previous study by George M et al. [12], who reported all the specimens treated by 120 seconds sodium hypochlorite showed a peak deproteinization and abundant exposed tubular porosities.

Specimens of thin smear layer groups showed higher scores as compared to those of thick smear layer groups. Thus, it can be speculated that the thick characteristics of smear layer may reduce the penetration of the NaOCl solution into dentin substrate and therefore less removal of collagen fibrils can be seen in demineralized dentin. In the aspect of bonding, this result also in line in the research that was conducted by Oliviera et al in 2005, as they mentioned thicker smear layers may negatively affect the ability of the self-etch system to penetrate through intact, mineralized dentin. Furthermore, some studies reported low bonding adaptation over thick dentin smear layers [10, 17].

The photomicrographs (Fig.3 and Fig.4) showed an increased number of morphological changes of dentin after the pre-treatment with NaOCl solution. After 15 seconds of pre-treatment (Group 3) and 30 seconds (Group 4) ,both showed evidence of less removal of the collagen network, providing the dentin surface with a more homogenous aspect compared with the demineralized dentin (Group 2). Apart from that, there were still number of collagen fibrils could be observed in those particular areas. Upon prolonged pre-treatment time (Group 5), this had triggered a greater number of collagen being removed (more than 1/3 of the specimen), which in some areas, the collagen were still present intact. By increasing the NaOCl pre-treatment duration up until 120 s, there were complete removal of the collagen with the presence of multiple porosities on dentin.

Therefore, the null hypothesis tested is to be rejected, since both factors of smear layer characteristics and sodium hypochlorite pre-treatment time had affected the ability of tissue dissolution and the dentin composition.

The morphological changes seen by the action of NaOCl pre-treatment, like those revealed in this study, could assist the clinicians to predict suitable pre-treatment methods to be conducted specifically in deep caries management cases since the procedure produces thicker smear layer preparation which may be less effective in terms of removing collagen fibrils network according to our results. However, this research may trigger another need for a study to be conducted in the future to determine whether the pre-treatment time may affect the bonding effectiveness of current adhesive systems available today.

Conclusion

Within limitations of this study, it can be concluded that sodium hypochlorite pretreatment had significantly affected the ultrastructural morphology of teeth substrate. Thin smear layer has better quality of smear layer and alteration of collagen fibrils as thin group had higher SEM score compared to thick smear layer group. Regardless of smear layer preparation, the longer the pretreatment time, the more removal of collagen fibrils can be observed.

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Author Contributions

All authors contributed toward data analysis , manuscript drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosure to declare.

Compliance with Ethical Standards

All procedures followed were in accordance with the ethical standards of the responsible committee. All institutional and national guidelines for the care and use of laboratory were followed. This article does not contain any studies with human or animal as test subjects.

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