Effect of Various Concentrations of Sodium Hypochlorite on Primary Dentin: An *in vitro* Scanning Electron Microscopic Study

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Background: *Sodium hypochlorite solutions have been evaluated for their effects in bonding procedures as they are found to deplete or remove the organic portion of the dentin, particularly the collagen fibrils. The* ***aim*** *of this in vitro study was to assess and compare the efficacies of 1%, 2.5%, 5% and 10% NaOCl at 30, 60 and 120s on etched primary dentin.* ***Methods:*** *84 primary anterior teeth were ground to expose a flat dentin area on the buccal surface. The specimens were divided into fourteen groups of six each based on the dentin surface treatment (35% phosphoric acid etching for 7 seconds-AE and/or NaOCl application), NaOCl solution concentrations (1%, 2.5%, 5% and 10%) and time of application (0, 30, 60 and 120s). Specimens were prepared for SEM and photomicrographs were taken of the surface and were scored against a five point scale, based on the smear layer and amount of collagen removed. The scores were submitted to Kruskal-Wallis and Mann Whitney tests.* ***Results:*** *This study showed the presence of smear layer in the control group. The group treated with Acid Etchant showed a demineralized pattern of dentin with exposure of dentin tubules and collagen fibrils network on the intertubular and peritubular dentin which was not significantly different from the groups treated with 1% and 2.5% NaOCl. Groups treated with 5% NaOCl were not statistically different from each other, the surface was corroded but collagen fibrils were not completely depleted. Groups treated with 10% NaOCl were not statistically different from each other and showed complete removal of collagen fibrils with wider tubular apertures and several secondary tubules on peritubular and intertubular dentin.* ***Conclusion:*** *Higher concentrations of NaOCl solutions (5% and 10%) produced significant changes in the etched primary dentin. The higher the concentration of the NaOCl solution, the lower can be the time for the application of the solution for the complete removal of collagen fibrils.*

Keywords: *NaOCl, SEM, primary dentin, deproteinization.*
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INTRODUCTION

Sodium hypochlorite solutions are widely used in various dental procedures based on their non-specific deproteinizing action. Such solutions have been routinely used for many years as part of chemo mechanical preparation procedures in endodontic preparation for root canal cleansing and shaping.[1]

In addition, sodium hypochlorite treatments have been evaluated for their effects in dentin bonding procedures[2] and for chemo-mechanical removal of carious lesions of dentin.

The dentin surface is acid etched prior to dentin bonding procedures to increase micromechanical retention and decrease marginal leakage[3] by removal of the smear layer,[4] which exposes a rough, high surface energy of collagen network.[5]

Dentin bonding procedure relies on the formation of a hybrid layer, which is formed by the polymerization of impregnated monomers into the exposed collagen of the demineralized (acid etched) dentin.[6-9] Some studies have shown that the hybrid layer is vital in increasing bond strength between the composite resin and dentin[8,10-14] while others have proved that it is responsible for microleakage, possibly because of the hydrolytic breakdown of exposed collagen. This is due to the formation of a weak zone of poorly impregnated monomers into the dentin collagen which is susceptible to breakdown by a long-term contact to water.[8,15,16]

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The dissolution of organic tissues by NaOCl solutions is based on the action of chloride over the proteins, forming chloramines, which are soluble in water. This reaction is directly proportional to the active chloride concentration present in the solution.[17] NaOCl solution alters the configuration or removes the organic component of dentin: especially the collagen fibrils.

The deproteinized dentin has higher hardness, modulus of elasticity,[1] wettability[18] and permeability[19] than the demineralized dentin. The dentin substrate is transformed, after deproteinization into a very porous structure with multiple irregularities and anastomoses, which could not be seen by normal demineralization process. However, depending on the adhesive system used dentin surface treatment with NaOCl can increase,[20] decrease[13] or not interfere[11] in bond strength between composite resin and dentin.

Although the efficiency of NaOCl on bonding procedures has been proved on permanent dentin, there is no standardization for NaOCl application parameters (concentration, time of application) on primary dentin in the literature.

Due to the morphological and constitutional differences of primary versus permanent teeth, they differ in their bonding. Deciduous teeth are more susceptible[21] to acid etching and it can be likely that the application of NaOCl to that substrate would produce different alterations when compared with permanent teeth.[2]

Therefore, the present study was undertaken to evaluate the alterations of etched dentin when subjected to different time and concentrations of sodium hypochlorite, using SEM. The hypothesis tested was that the higher the concentration of the sodium hypochlorite solution, the lower the time of application of this solution for total depletion of exposed collagen fibrils.

Aim of the study: 1) To evaluate the alterations of etched primary dentin when subjected to different time and concentrations of sodium hypochlorite. 2) To compare the efficacies of 1%, 2.5%, 5% and 10% concentrations of sodium hypochlorite. 3) To compare the effectiveness of sodium hypochlorite at 30, 60 and 120 s.

MATERIALS AND METHOD

Eighty-four primary anterior primary teeth, which were extracted for therapeutic purposes were taken for the study. They were stored in 0.5% Chloramine T solution, at 4 degrees centigrade, for less than a week.[26] The root or root remains of all the teeth were removed 1mm below Cemento enamel Junction (CEJ) using a water cooled diamond disc. A flat dentin area on the buccal surface was also exposed. The specimens were then examined under a 25x magnification for any remaining enamel, with a stereomicroscope (Zeiss, Manaus, AM, Brazil). Commercially available 1%, 2.5%, 5% and 10% NaOCl solutions were also used for the study. 35% Phosphoric Acid Gel Etchant (Ultradent) was used. The specimens were randomly divided into fourteen groups of six teeth each based on treatment of the substrate-acid etching and/or NaOCl application according to the groups assigned as follows:

Group 1 - G1 - Control Group	No treatment
Group 2 - G2	Acid Etching only
Group 3 - G3	Acid Etching + 1%NaOCl for 30s
Group 4 - G4	Acid Etching + 1%NaOCl for 60s
Group 5 - G5	Acid Etching + 1%NaOCl for 120s
Group 6 - G6	Acid Etching + 2.5%NaOCl for 30s
Group 7 - G7	Acid Etching + 2.5%NaOCl for 60s
Group 8 - G8	Acid Etching + 2.5%NaOCl for 120s
Group 9 - G9	Acid Etching + 5%NaOCl for 30s
Group 10 - G10	Acid Etching + 5%NaOCl for 60s
Group 11 - G11	Acid Etching + 5%NaOCl for 120s
Group 12 - G12	Acid Etching + 10%NaOCl for 30s
Group 13 - G13	Acid Etching + 10%NaOCl for 60s
Group 14 - G14	Acid Etching + 10%NaOCl for 120s

No treatment was done on G1 specimens. 35% Phosphoric acid gel was applied for 7s on the dentin surface of the specimen, copiously rinsed for 15s with water using a 25 gauge syringe and blot dried for the rest of the groups. The specimens were irrigated with 1%, 2.5%, 5% and 10% NaOCl solution application for 30, 60,120 s each using 25 gauge syringes, according to the distribution of the groups from G3 to G14. The specimens were finally copiously rinsed with distilled water in a continuous flow for 30 s with a 28 gauge syringe and carefully dried.

All the specimens were immersed in SEM fixative (2% glutaraldehyde) to preserve the dentin surface for SEM analysis. The specimens were rinsed with saline and sequentially dehydrated in 10, 25, 50, 75, 90 and 100% alcohol at 15 minutes intervals. Specimens were then mounted on metal stub and then ion sputtered in an ion sputtering device (Fine Coat, JEOL, JFC- 1100 E, JEOL Technics Co; Tokyo, Japan) for 5 min, before viewing under Scanning Electron Microscope (JEOL, JSM 840A, JEOL Technics Co; Tokyo, Japan).

After a general survey of the entire buccal surface, photomicrographs were taken at 1000X and 3000X magnification of each of the specimens. Images were captured for scoring. Images of each specimen were read by one examiner who was an experienced clinical academic with no knowledge of sample group or treatment and a single score was given against the following five-point scale, described below.

Score 0	Presence of smear layer SL
Score 1	Absence of SL and non-altered collagen Fibrils (no region of the specimen present removal of collagen network)
Score 2	Absence of SL and collagen Fibrils slightly altered (less than 1/3rd of collagen Fibrils is removed)
Score 3	Absence of SL and collagen Fibrils severely altered (more than 1/3rd of collagen Fibrils is removed)
Score 4	Absence of SL and complete removal of collagen Fibrils (The dentin surface has a much corroded aspect with several porosities on the intertubular dentin).

There was no restriction on time taken to read each image. A break of 5 minutes was taken after reading each image. The final data was analyzed statistically using Kruskal Wallis test and Mann Whitney test.

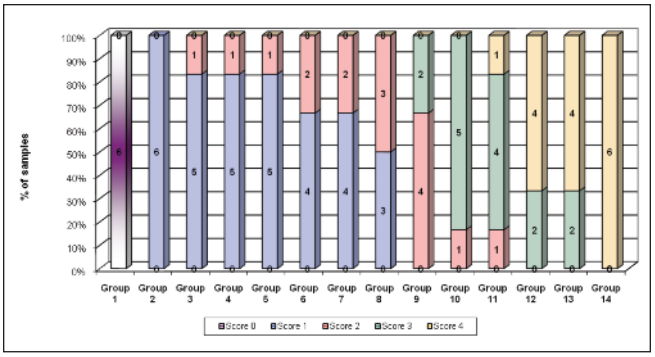
RESULTS

Mean, standard deviations and Median scores for each group are shown in Table 1 & Graph 1. Comparison of mean scores of the fourteen groups is shown in Graph 2. Comparison of median scores of the fourteen groups is shown in Graph 3.

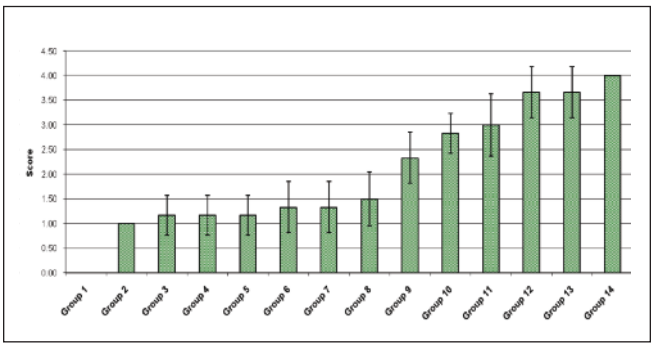
Table 1. Descriptive Statistics and Kruskal Wallis Test Result

Group*	Mean	Std dev	Min	Median	Max	Kruskal-Wallis Chi-Square	P-Value
Group 1	0.00	0.00	0.00	0.00	0.00	73.154	<0.001
Group 2	1.00	0.00	1.00	1.00	1.00		
Group 3	1.17	0.41	1.00	1.00	2.00		
Group 4	1.17	0.41	1.00	1.00	2.00		
Group 5	1.17	0.41	1.00	1.00	2.00		
Group 6	1.33	0.52	1.00	1.00	2.00		
Group 7	1.33	0.52	1.00	1.00	2.00		
Group 8	1.50	0.55	1.00	1.50	2.00		
Group 9	2.33	0.52	2.00	2.00	3.00		
Group 10	2.83	0.41	2.00	3.00	3.00		
Group 11	3.00	0.63	2.00	3.00	4.00		
Group 12	3.67	0.52	3.00	4.00	4.00		
Group 13	3.67	0.52	3.00	4.00	4.00		
Group 14	4.00	0.00	4.00	4.00	4.00		

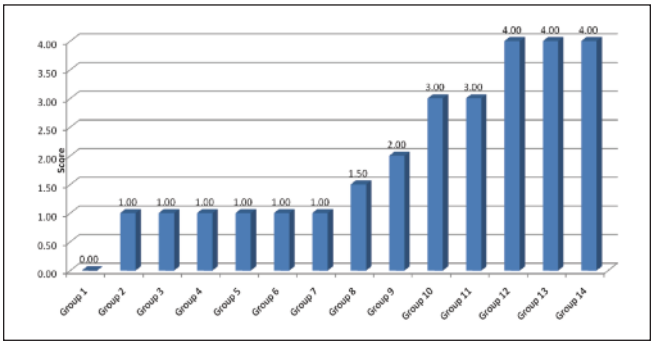
*Group 1-no treatment, Group 2-Acid Etching, Group 3-1%NaOCl for 30s, Group 4-1%NaOCl for 60s, Group 5-1%NaOCl for 120s, Group 6-2.5%NaOCl for 30s, Group 7-2.5%NaOCl for 60s, Group 8-2.5%NaOCl for 120s, Group 9-5%NaOCl for 30s, Group 10-5%NaOCl for 60s, Group 11-5%NaOCl for 120s, Group 12-10%NaOCl for 30s, Group 13-10%NaOCl for 60s, Group 14-10%NaOCl for 120s.



Graph 1. Distribution of Scores across Groups



Graph 2. Mean Scores Recorded in the Groups



Graph 3. Median Scores Recorded in the Groups

From Table 1, it was observed that there was a statistically significant difference between the groups with respect to the median score (P<0.001). Higher mean and median scores are found in all Groups treated with 10% NaOCl. The next higher mean and median score is found in all Groups treated with 5%NaOCl. These groups are next followed by Group treated with 2.5% NaOCl for 120s. The mean and median scores of Groups treated with 2.5% NaOCl for 30, 60s come next in order and both of them have an equal mean and median score. Also, the mean and median scores of samples treated with 1% NaOCl are found to be equal. The Group treated with Acid etchant recorded a lower mean score compared to other groups except the group that was not treated, which recorded the lowest mean and median score.

Further pair-wise multiple comparisons using Mann-Whitney Tests showed that all specimens of the control (G1) showed the presence of smear layer, which was significantly different from the other groups. The Group treated with Acid Etchant showed a demineralized pattern of dentin with exposure of dentin tubules and collagen fibrils network on the intertubular and peritubular dentin which was not significantly different from the groups treated with 1% and 2.5% NaOCl.

Groups treated with 5% NaOCl were not statistically different from each other and typically showed wide tubular apertures with presence of secondary tubules. The surface was corroded but collagen fibrils were not completely depleted.

Groups treated with 10% NaOCl were not statistically different from each other and showed complete removal of collagen fibrils with wider tubular apertures and several openings of secondary tubules.

DISCUSSION

Several morphological and compositional differences in dentin of permanent and primary teeth exist.[22] Sumikawa *et*

al[23] observed significant microstructural differences of primary and permanent teeth and the presence of microcanals were a common finding at all depths of dentin, which would clinically decrease the area of dentin available for bonding procedures.

NaOCl has both disinfecting and non specific deproteinizing actions[1] and thus has various uses in the dental set up. It has been used in procedures like chemomechanical treatment in endodontic preparations, chemomechanical removal of carious lesions in dentin (Cariosolv) and in dentin adhesion procedures.[2,6,9-11,24,25-28] When the dentin surface is treated with NaOCl, it can increase,[6,24,25-27,29] decrease[9,13] or not interfere[2,11,29] in the bond strength between the composite resin and dentin, depending on the adhesive system used.

When dentin is treated with NaOCl, it causes dissolution of the collagen fibrils increasing hardness, modulus of elasticity,[1] wettability[18] and permeability,[19] which facilitates easier access of resin into the substrate.[28] In order to achieve these advantages, complete removal of collagen has to be obtained. Unexpected, deleterious reactions between resin and dentin have been noted if altered collagen fibrils remain.[30]

The action of NaOCl depends on the active chloride content present in solution at the time of application.[31] Our study incorporated NaOCl as a solution that was prepared the same day to prevent the loss of chloride.

In our study, all concentrations of sodium hypochlorite after demineralization had significant alterations on the microstructure of primary dentin. Although the lower concentrations of NaOCl (1%, 2.5%) did not considerably modify the morphology of demineralized primary dentin, 5% and 10% NaOCl solutions made remarkable alterations to the morphology of demineralized primary dentin.

When the specimens were not subjected to any treatment (G1), dentinal tubules were completely absent and showed the presence of a smear layer (score: 0) [Figure 1.]. Similar aspects of a permanent demineralized dentin can be achieved on a primary tooth using 35% phosphoric acid for 7s than the 15s that is usually applied for permanent dentin. The specimens of G2 which underwent only acid etching with 35% phosphoric acid solutions for 7s showed clearly the openings of the dentinal tubules and collagen fibrils network (score: 1) [Figure 2.]. The results are in comparison with the previous study done by Correr *et al,*[32] where they also observed similar results.

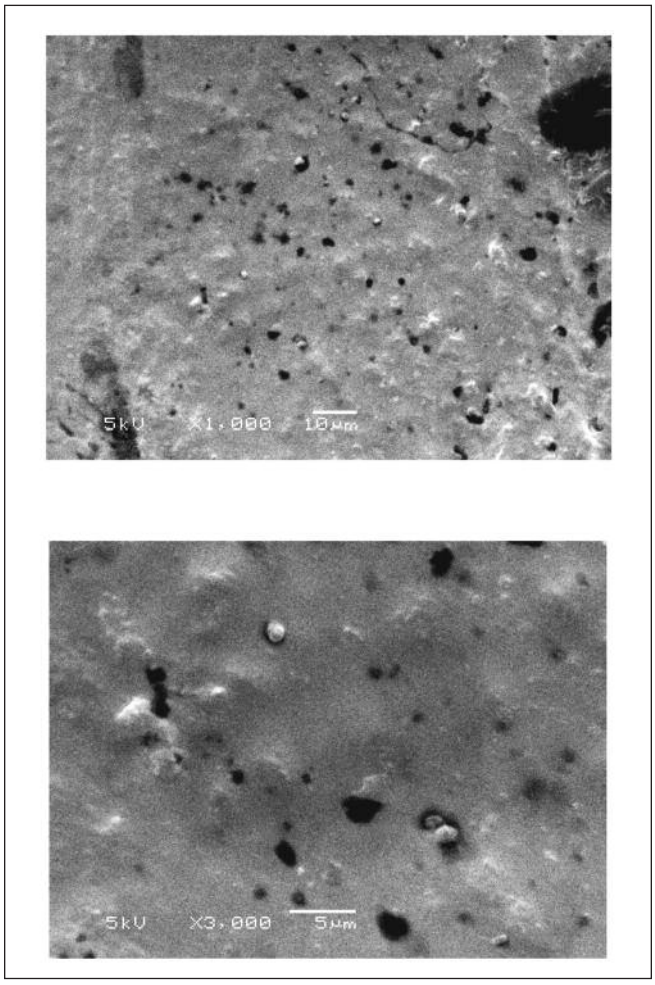


Figure 1. Typical dentin surface with no substrate treatment showing smear layer and no dentin tubules.

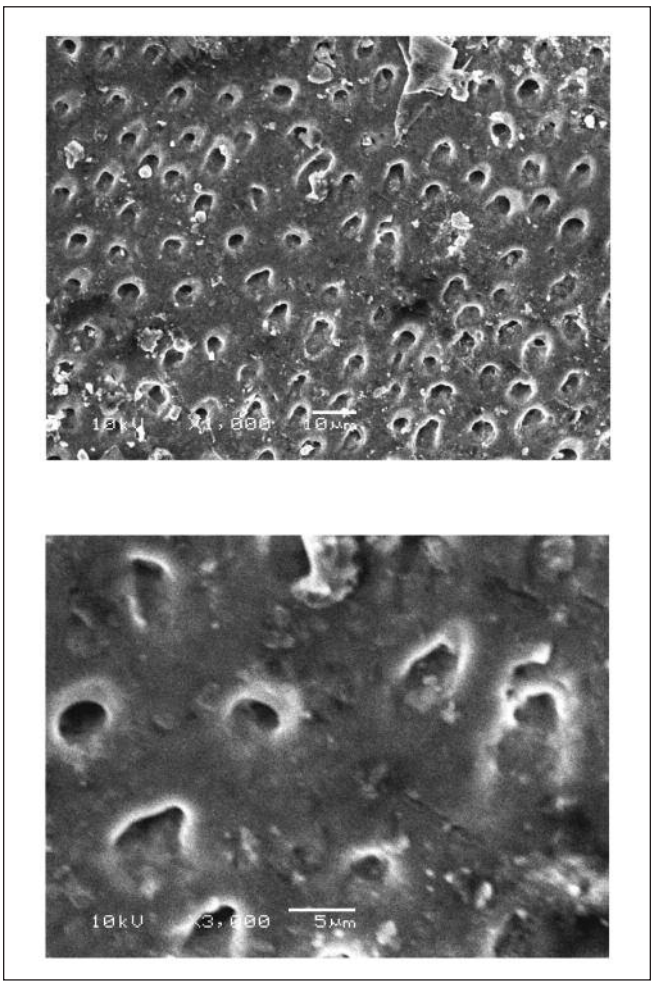


Figure 2. Typical topography of demineralised dentin(G2) showing open dentin tubules and exposed collagen layer. Scored 1.

When using 1%, 2.5% NaOCl solutions and 5% NaOCl solutions for 30s on demineralized dentin, we found that Group 3 to Group 9 showed least evident superficial collagen alterations and there was a very slight increase in the apertures of the tubules when compared with G2 (score: 2) [Figure 3.].This is also in correlation to the previous study by Correr *et al*,[32] where they found similar results from 5% NaOCl for 30s although they did not test the lower concentrations of NaOCl.

When 5% NaOCl was tested for 60 and 120s, the samples showed no significant difference between the two groups, but were significantly different from all the other groups. The changes were more evident than in the previous groups, the tubule apertures were wider and the surfaces were corroded but collagen was not completely depleted (score: 3) [Figure 4.].

When 10% NaOCl was used for 30, 60 and 120s, the samples was observed to have a more corroded surface, with wider tubular apertures and several secondary tubules when compared to the previous groups (score: 4) [Figure 5.].

This study suggests that 10% NaOCl is most effective in the complete removal of collagen in primary dentin, following acid etching with 35% phosphoric acid gel and 5% NaOCl showed comparable potential when used for 120s. Also, our study showed that 1% and 2.5% NaOCl is not effective in the complete removal of collagen. The alterations in the dentin micro morphology were progressive according to the concentration and the time of application of the NaOCl, when compared with the control group (Group 1) and the alterations promoted by the acid etching group (Group 2). Our results corroborate with the findings of the previous study by Correr *et al,*[32] which tested only 5% and 10% NaOCl solutions in primary teeth and not the weaker concentrations of sodium hypochlorite that is more commonly available in the pediatric dental clinic.

The results of this study also match those of Di Renzo *et al*[33] who concluded that the alterations from sodium hypochlorite are time dependent. They used Fourier transform infrared spectroscopy (FTIRS) to show the gradual removal of collagen fibers following 12% NaOCl treatment over 2 minutes.

Other studies also established the complete removal of collagen by NaOCl treatments in different concentrations and time of action. Perdigao *et al*[9] also confirmed the complete removal of collagen with 5% NaOCl in 120s. However, Osario *et al,*[30] showed that the same procedure did not

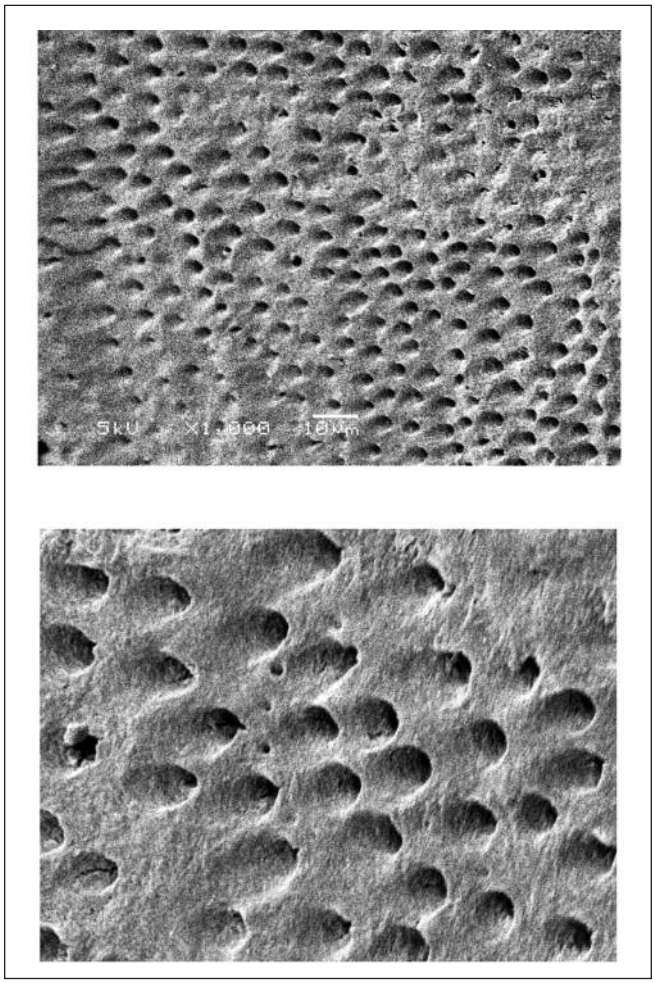


Figure 3. Typical topography of deproteinised dentin (G8) after application of 2.5% NaOCl for 120s showing superficial collagen alterations and increase in the apertures of the tubules. Scored 2.

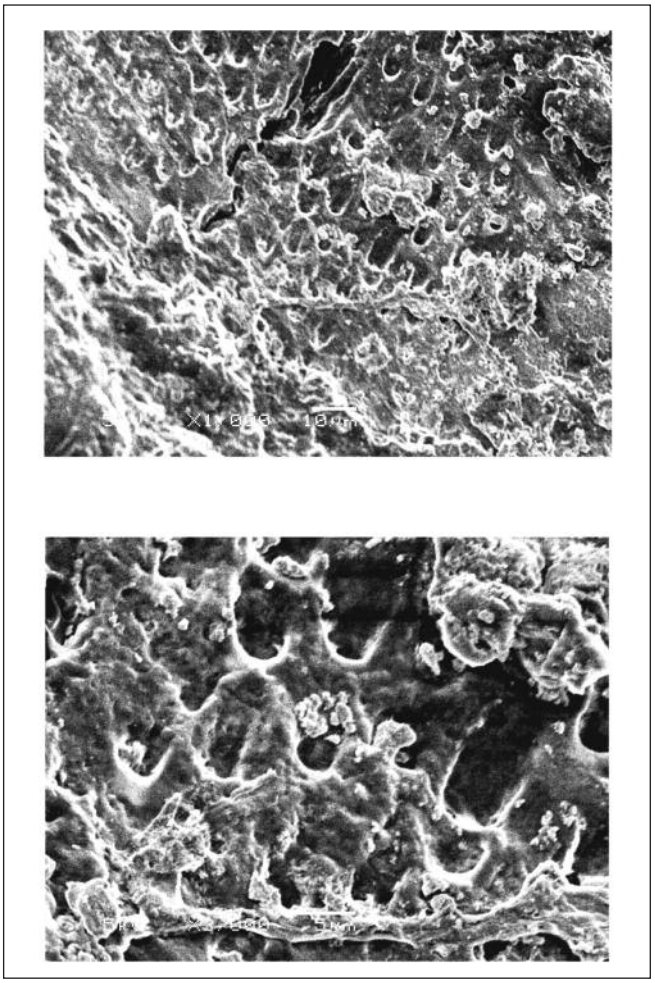


Figure 4. Typical topography of deproteinised dentin (G11) after the application of 5% NaOCl for 120s showing a corroded surface. Scored 4.

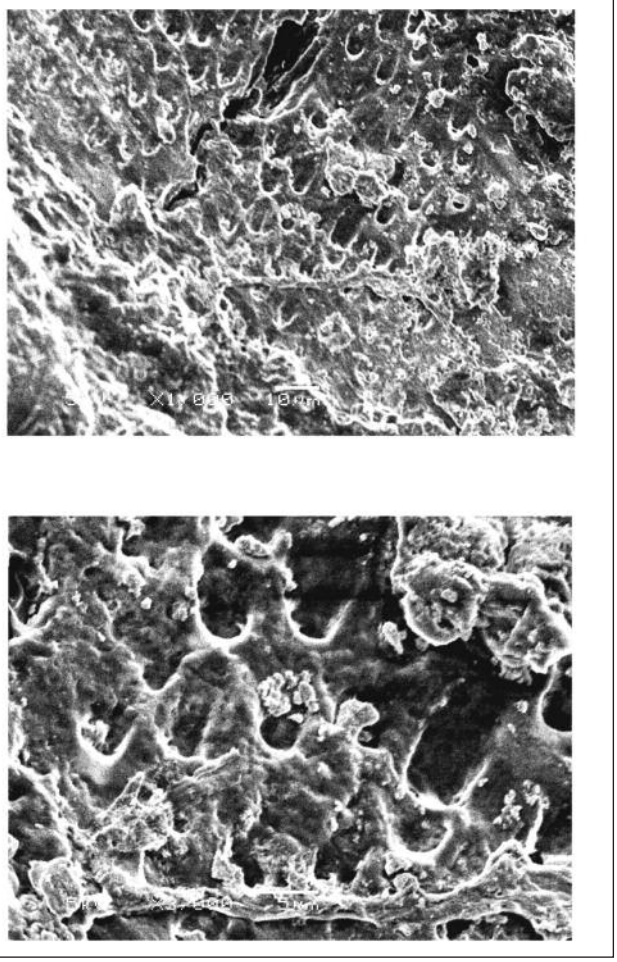


Figure 5. Typical topography of deproteinised dentin (G14) after the application of 10% NaOCl for 120s showing a corroded surface with wide openings of secondary tubules. Scored 4.

remove the collagen fibrils completely, leaving a hybrid layer in some parts of the sample. These could be related to the delivery system of the hypochlorite solution (gel or solution), the chloride content of the solution and the depth and organic content of the dentin.[34]

The difference in the collagen removal using the same protocol between permanent and primary dentin could be because of the difference in dentin morphology and composition.

The alterations provoked in the study, could produce a more predictable substrate for bonding. In the absence of collagen fibrils soaked in water, the need for a wet bonding protocol would be questionable. Also a porous and mineral rich substrate, chemically similar to unaltered dentin, should allow good mechanical retention and also provide the possibility of chemical bonding for future bonding systems.

Based on the results of this study on the deproteinization protocols in primary dentin, with the complete removal of collagen, the time for application of 5% NaOCl solutions must be prolonged and for 10% NaOCl solutions, the time can be reduced to 30s.

All specimens of the control (G1) showed the presence of smear layer. The Group treated with Acid Etchant showed a demineralized pattern of dentin with exposure of dentin tubules and collagen fibrils network on the intertubular and peritubular dentin which was not significantly different from the groups treated with 1% and 2.5% NaOCl.

Groups treated with 5% NaOCl were not statistically different from each other and typically showed wide tubular apertures with presence of secondary tubules. The surface was corroded but collagen fibrils were not completely depleted.

Groups treated with 10% NaOCl were not statistically different from each other and showed complete removal of collagen fibrils with wider tubular apertures and several openings of secondary tubules.

CONCLUSIONS

Within the limits of the present *in vitro* study we conclude the following:

A progressive increase in the removal of collagen on the etched dentin surface occurs as the concentration of NaOCl solution is increased.

1% and 2.5% sodium hypochlorite solutions do not produce significant alterations on the microstructure of etched primary dentin. 5% and 10% sodium hypochlorite solution application produces a significant and complex alteration on the microstructure of etched primary dentin.

The higher the concentration of the NaOCl solution, the lower can be the time for the application of the solution for the complete removal of collagen fibrils.

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