

Effects of NaOCl or Combination of NaOCl and Various Acids on Dentin Surface Morphology: A SEM Study

SUMMARY

The aim of this study was to evaluate by SEM morphological changes of dentin surfaces under the use of 5% NaOCl before applying different acids for etching. In the study, dentin surfaces were prepared from the middle third of 20 non-carious human third molars. Samples were divided into 2 groups with 3 subgroups each. In the first group, no application was performed on dentin surfaces. In the second group, 5% NaOCl was applied for 60 seconds on the dentin surfaces then rinsed with distilled water for 5 seconds. In all of the subgroups, dentin surfaces were etched by different conditioning agents for 15 seconds then rinsed for 30 seconds. After surface applications, all of the specimens were placed in distilled water. Morphological changes of dentin surfaces were determined by SEM. The results were evaluated by the scoring system based on Brannström et al.

In the NaOCl treated groups, scores were as follows: The score of dentin without NaOCl treatment was 0, while it was 4 after phosphoric acid application, 3 after only citric acid-ferric chloride, and 1 after only maleic acid application. In the group where only NaOCl was applied, the score was not exactly 1 but slightly less. The score for NaOCl with phosphoric acid was 4, NaOCl with citric acid-ferric chloride was 3, and NaOCl with maleic acid was 2. Weak acid was found more effective on NaOCl treated dentin surfaces when compared with non-treated dentin surfaces.

Keywords: Dentin; Sodium Hypochlorite; SEM; Maleic Acid; Phosphoric Acid

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Introduction

Prognosis of root canal treatment depends on the success of the final restoration, because penetration of microorganisms from a coronal direction may re-infect the root canal system¹. So, the quality of the coronal restoration is a crucial factor for the prognosis of the treated tooth after the root canal treatment². Recent years, parallel to the development of dentin bonding systems, adhesive restorations are mostly preferred for restoration of endodontically treated teeth.

Also, there is a widely held belief that root-treated teeth are weakened and more susceptible to fracture than vital teeth^{3,4}. Several factors, such as the presence of an endodontic access cavity, wide coronal flaring of canals, altering the properties of dentin related to the

loss of pulp vitality can cause weakening the root-treated teeth⁵. In addition to these factors, intra-canal irrigants, medicaments and materials may contribute to physical and mechanical properties of dentin. For example, eugenol-containing root canal sealers can harden intra-canal dentine⁶, while chloroform, xylene and halothane soften dentine⁷. Sodium hypochlorite (NaOCl) is widely used as a root canal irrigant because of its antibacterial properties, and also its ability to denature toxins^{8,9}. NaOCl application results in gross debridement, lubrication, dissolution of tissues, removal of the collagen layer, and dehydration of the dentin¹⁰. However, it was observed that the NaOCl solutions can not remove the smear layer from the root canal walls completely¹¹. Several researchers¹²⁻¹⁶ have studied the role of NaOCl in dentin permeability and dentin adhesion. Depending on each testing method and

specific composition of each dentin adhesive, application of NaOCl upon etching may increase or decrease bond strengths^{14,17-21}. Dentin is composed of approximately 22% organic material by weight. Most of this organic structure consists of collagen, which exerts a considerable contribution to the mechanical properties of dentin. Therefore, NaOCl has the possible ability to weaken dentine. Various forms of surface morphology changes may occur depending on the type of acid applied to dentin surfaces²²⁻²⁴.

The aim of this study was to evaluate morphological changes of dentin surfaces due to the application of various acids with, or without utilization of NaOCl.

Materials and Methods

Non-cariou human third molars, stored in distilled water after extraction, were used in this study. Dentin surfaces were prepared from the coronal middle-third of the teeth. A high-speed dental drill with a tapered diamond bur cooled by water was used to form 3 mm thick dentin discs. All of the dentin discs were then ground on 600 grit SiC paper to reduce their thickness to 1 mm and produce a smear layer.

The study was planned in 2 main groups, which were divided in 1 control and 3 subgroups related to application of different agents on dentin (Tab. 1). In the first group, only conditioning agents (37% phosphoric

acid, 10% citric acid + 3% ferric chloride, 10% maleic acid) were applied on dentin surfaces. In the second group, 5% NaOCl was applied for 60 seconds on all of the dentin surfaces and then rinsed with distilled water for 5 seconds. After NaOCl application, dentin surfaces were etched by the different conditioning agents in all of the subgroups - by acid gels for 15 seconds then washed with a water-air spray for 30 seconds. After surface applications, all of the specimens were placed in distilled water. The effects of the conditioning agents on dentine surfaces were evaluated by using SEM (Jeol 6335 F, Jeol Ltd., Tokyo, Japan) under x2000 magnification. The operating voltage for SEM observations was 20kV at a working distance of 25 mm. SEM micrographs were scored blind for their effects on the smear layer. Scores from 0 to 4 were rated by 3 separate assessors, and the median score was used to produce a ranking order for the conditioning agents tested. The scoring system was based on that of Brannström et al²⁵.

0 = surface completely covered with thin smear layer, no tubules visible;

1 = surface covered with thin smear layer but orifices of tubules visible; occasional tubules open;

2 = smear layer partly removed and orifices of most tubules open or partially open;

3 = smear layer mainly removed, most tubules completely open;

4 = smear layer completely removed and peritubular dentin removed, resulting in increased size of tubular orifices.

Table 1: Groups (main and subgroups) used in the study.

| Group 1 | Group 1A | Group 1B | Group 1C |
|----------------------------|---|--|---|
| No application of 5% NaOCl | 37% phosphoric acid is applied for 15 seconds only | 10% citric acid + 3% ferric chloride is applied for 15 sec. | 10% maleic acid is applied for 15 seconds only |
| Group 2 | Group 2A | Group 2B | Group 2C |
| 5% NaOCl is applied | After the NaOCl application, 37% phosphoric acid is applied for 15 sec. | After the NaOCl application, 10% citric acid + 3% ferric chloride is applied for 15 sec. | After the NaOCl application, 10% maleic acid is applied for 15 sec. |

Results

SEM micrographs of Group 1 and Group 2 are shown in figures 1 and 2. In general, topographical views of dentin surfaces treated by only strong acids (phosphoric acid and ferric chloride/citric acid) revealed similar features at the same magnifications (Figs. 3 and 5). But SEM findings of dentine surfaces treated by weak

acid (maleic acid) were found to be different from other micrographs (Fig 7).

On the other hand, after application of NaOCl alone on dentin surfaces, the effectiveness of NaOCl was found to be very weak on the removal smear layer (Figs. 1 and 2). Following utilization of this irrigant, dentin surfaces were covered with a thin smear layer, but orifices of tubules could still be distinguished (the score was not exactly 1 but slightly less). Acid application after NaOCl significantly enhanced the removal of

smear layer. When topographical appearances of dentin surfaces were evaluated, it was observed that application of both phosphoric acid alone (Group 1A - Fig. 3) or in combination with NaOCl (Group 2A - Fig. 4) produced the same score of 4. Similarly, in the group where citric acid/ferric chloride were applied, the score was 3,

regardless NaOCl (Groups 1B and 2B - Figs. 5 and 6). Since maleic acid is weaker than the others, after using this conditioner alone (Group 1C - Fig. 7), the surface score was 1. However, when maleic acid was applied after NaOCl (Group 2C - Fig 8), topographical appearance of the surface was scored 2.

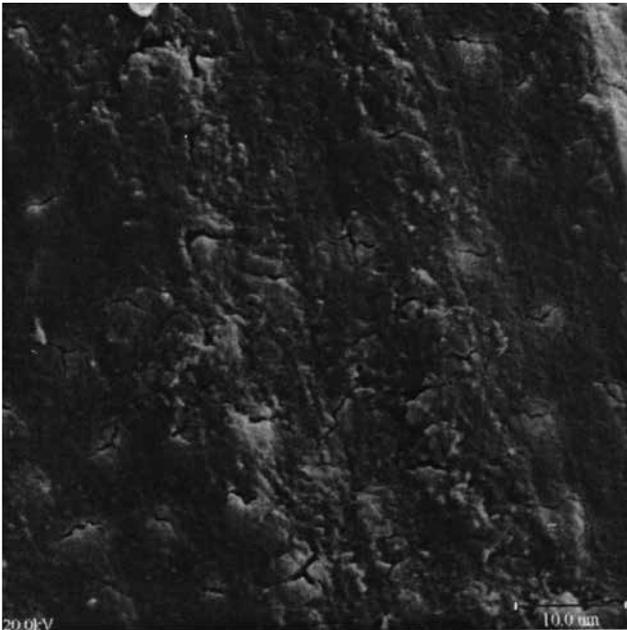


Figure 1: The non-treated dentin surface microphotograph

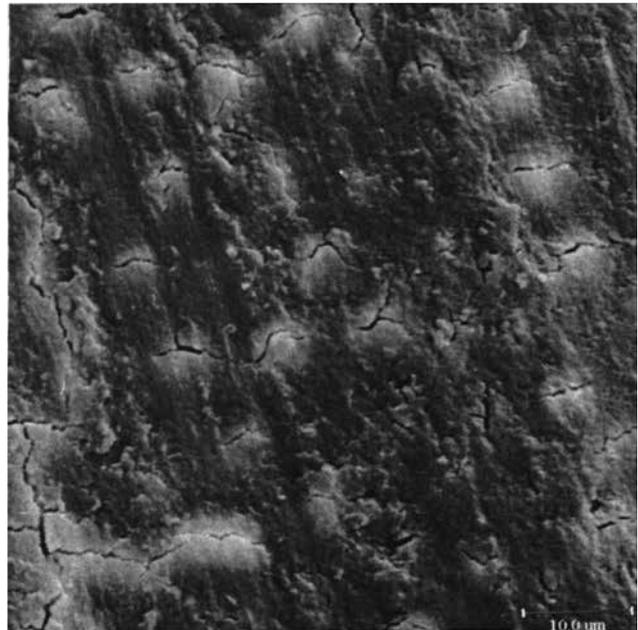


Figure 2: Dentine surface microphotograph when treated with 5% NaOCl

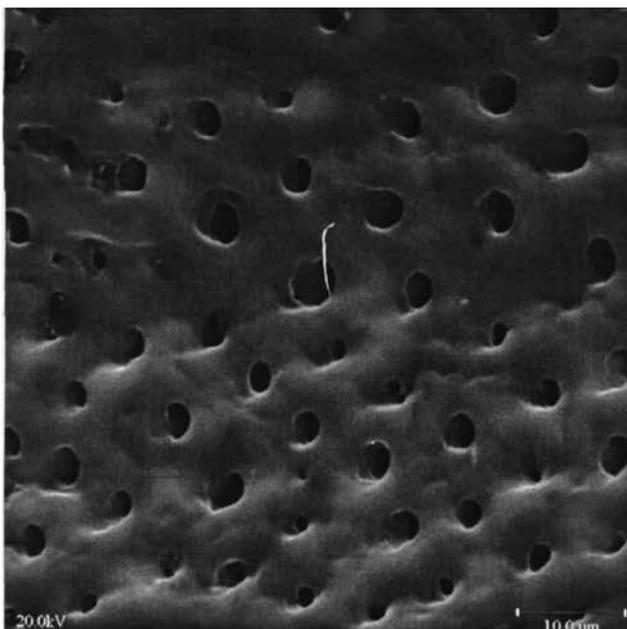


Figure 3: Dentine surface microphotograph when treated with 37% phosphoric acid only

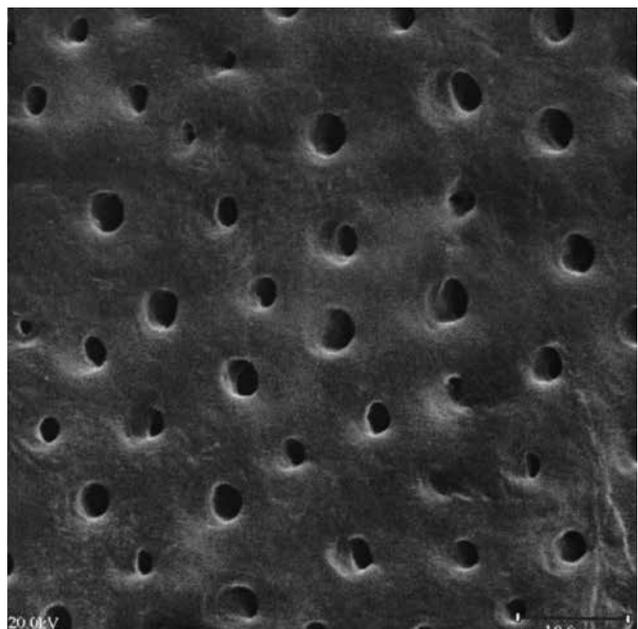


Figure 4: Dentine surface microphotograph when treated with 5% NaOCl and 37% phosphoric acid

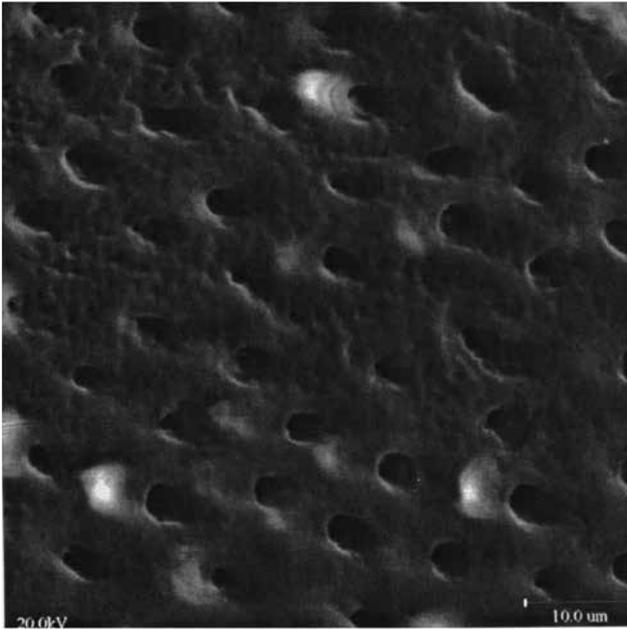


Figure 5: Dentine surface microphotograph when treated with 10% citric acid + 3% ferric chloride

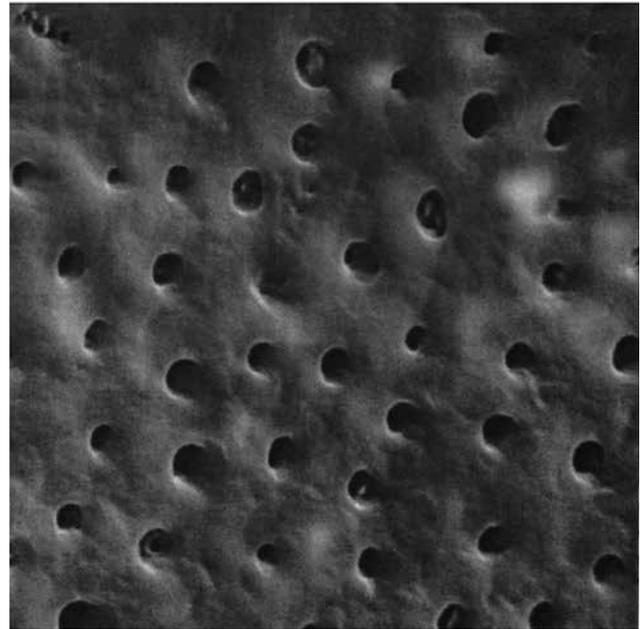


Figure 6: Dentin surface microphotograph when treated with 5% NaOCl and 10% citric acid + 3% ferric chloride

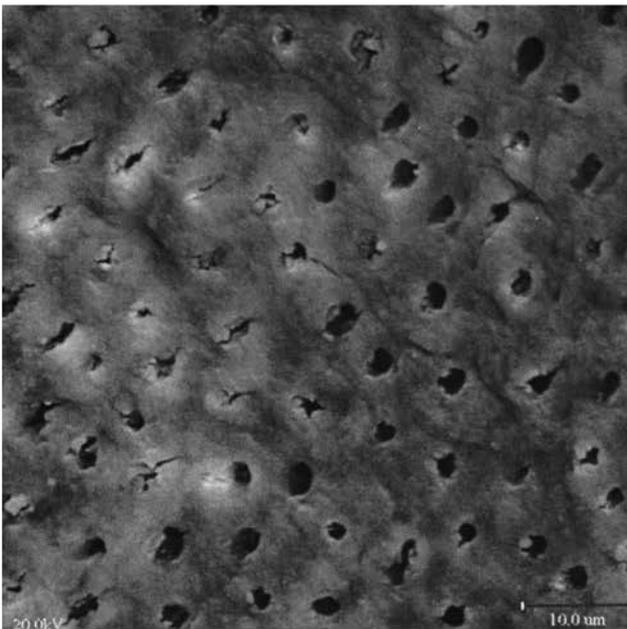


Figure 7: Dentine surface microphotograph when treated with 10% maleic acid only

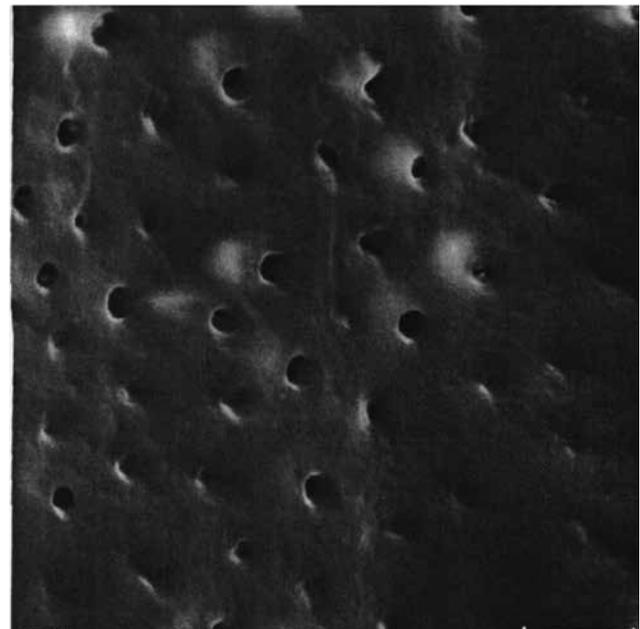


Figure 8: Dentine surface microphotograph when treated with 5% NaOCl and 10% maleic acid

Discussion

In this study, the effect of NaOCl only or NaOCl / acid solution combinations on coronal dentin surfaces were evaluated. NaOCl is routinely used for irrigation during endodontic treatments. So the effect of this irrigant on organic structure of dentin and the ultra-structural

views of this effectiveness are important to understand the micro-retention potential of dentin surface treated by NaOCl. In addition to this, various acid solutions are used before restorative procedures and they are applied to dentin surfaces irrigated with NaOCl. For these reasons, investigating the effects of NaOCl/various acid solution combinations on dentin surfaces and confirmation of this

effect by SEM microphotographs is interesting. Although, NaOCl irrigant is applied only inside root canals during endodontic treatment, the solution affects lateral walls of the pulp chamber and dentin surfaces of the crown cavities as well.

Various acid solutions that are strong or weak are used for acid-etching procedures. For example, phosphoric acid and citric acid/ferric chloride etching solutions are strong acids but maleic acid is weak^{26,27}. In addition to this, demineralization degree of acid solutions changes related to pH levels of etching solutions²⁸⁻³¹.

The results of this study showed that scores of dentin surfaces prepared by phosphoric acid or citric acid/ferric chloride remained similar regardless the application of NaOCl before acid etching (for phosphoric acid: score 4; for citric acid/ferric chloride: score 3). But ultra-structural views of dentin surfaces prepared by maleic acid and irrigated by NaOCl, or non-irrigated before were found to be different (score 2 for NaOCl with maleic acid and score 1 for only maleic acid). These results show that the application of NaOCl on dentin surface before etching procedure is more important for weak acids than for strong acids, because NaOCl irrigation enhances the etching effects of weak acids on dentin surfaces.

The results of previous studies showed that chemical irrigants, such as NaOCl, are commonly used as nonspecific proteolytic agents capable of removing organic material in endodontic treatment^{23,32}. According to the studies about concentrations of NaOCl, 5% was the most effective in killing bacteria established within dentinal tubules. Therefore, 5% NaOCl is generally preferred as an endodontic irrigant during root canal treatments³³⁻³⁵.

After endodontic treatment, generally, there is a significant amount of hard tissue loss and adhesive systems may be more advantageous in these cases. Preparation of tooth surfaces after root canal treatments is important to obtain appropriate dentine surface for successful hybridization.

NaOCl application also results in destruction of microorganisms, dissolution of tissues, removal of the collagen layer, and dehydration¹⁰. On the other hand, dentin contains 22% organic material, mainly collagen type I, which plays a major mechanical role³⁶, and it was reported that depletion of the organic phase after NaOCl treatment may cause mechanical change³⁷. Even though, the ultra-structural views and scores of the dentin surfaces treated by NaOCl and etched with strong acids (phosphoric acid or citric acid/ferric chloride) were found to be practically the same, mechanical properties of dentin surfaces irrigated by NaOCl might be weaker than non-irrigated groups³⁸. However, different scores were obtained for maleic acid groups depending upon NaOCl irrigation (score 2 for

irrigated group and score 1 for non-irrigated group). But acid-etching with weak acid after NaOCl irrigation did not cause any destruction on dentin surface (score 2). According to the results of the present study, it can be speculated that although mechanical changes of dentin surface occur due to NaOCl irrigation, the additional negative effects of weak acids on mechanical properties of dentin surfaces can be less than strong acids.

Yiu et al³⁹ reported that sodium hypochlorite remained within the porosities of mineralized dentin after its application during root canal treatment. This residual irrigant solution in mineralized dentin may cause the diffusion of etching solutions to deeper layers of mineralized tissue, so etching solutions may be more effective on mineralized structure.

It is known that phosphoric acid or citric acid/ferric chloride etching agents are effective on dentin tissue and they can create suitable conditioned surfaces for successful bonding between resin and dentin^{29,40}. In this study, scores of dentin surfaces (irrigated or non-irrigated) and etched by these acids were found to be almost the same (score 4 for phosphoric acid and score 3 for citric acid/ferric chloride). According to these results, it can also be speculated that NaOCl application does not decrease the bonding strength between adhesives and dentin surfaces.

In previous studies, it was found that 5% of NaOCl produced significantly large reductions in resin-dentin bond strengths especially for dentin surfaces etched by phosphoric acid or citric acid/ferric chloride^{35,41-44}. On the other hand, Öztürk and Özer⁴¹ reported that after NaOCl application on dentin surfaces, self-etching bonding systems with low pH^{45,46} were more successful than the other systems with high pH in bonding to dentinal wall. Accordingly, adhesive systems that contain weak acids or low acidulated primers are more suitable alternatives for the preparation of dentin surfaces treated with NaOCl³⁸. When strong acids are applied on dentin surfaces after NaOCl irrigation, more destruction can be created. This has been confirmed by SEM observation, possibly due to residual irrigant solution in mineralized dentin³⁹. Utilization of weaker acids for conditioning dentin surfaces previously irrigated with NaOCl might be more suitable to obtain higher and more successful bonding strength between resin and dentin surfaces. Further *in vitro* bonding studies are necessary to evaluate effectiveness of various acids (strong, weak acids) on dentin surfaces irrigated with NaOCl.

Conclusion

If an acid agent will be used on teeth previously treated with NaOCl, weak acids can be preferred to etch the dentin surfaces instead of strong acids.

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