Dentin Topographic Features following Chemomechanical Caries Removal in Primary Teeth

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Aim: *Study the topographic features of dentin after caries removal with a chemomechanical agent (Papacarie) compared with the conventional drilling method.* ***Study design:*** *The sample included 7 exfoliated and extracted primary teeth with carious dentin lesions, not reaching the pulp. Each tooth was sectioned longitudinally through the center of the carious lesions into two halves. The teeth were then divided into two groups according to the method of caries removal. Following caries removal, dentin topography and the cut section were examined using the scanning electron microscope.* ***Results:*** *Papacarie produced an irregular, porous, rough and globular dentin appearance. The dentin surfaces were generally free of smear layer, visible bacteria and the dentinal tubules were opened. The dentin cut surfaces showed patent dentinal tubules with open orifices. The drilling method created a smooth and amorphous surface with a continuous smear layer occluding the dentinal tubules. Numerous bacteria were also observed. The cut dentin surfaces showed patent dentinal tubules with their orifices plugged with smear layer.* ***Conclusions:*** *Papacarie produced a rough and porous surface with partial or complete removal of the smear layer and opened dentinal tubules, while the drill produced a smooth surface with uniform smear layer occluding the dentinal tubules.*

Key words: *Chemomechanical caries removal, dentin caries, primary teeth, Papacarie.*

INTRODUCTION

The chemomechanical caries removal (CMCR) technique gained interest in dental researches due to its concept of tissue preservation while insuring removal of the denatured dentin collagen. Chemomechanical caries removal systems act by causing degradation of the partially degraded collagen in the outer layer of infected dentin [1].

Carisolv was introduced in Europe in 1998. Studies proved its efficiency in caries removal [2, 3]. However, Carisolv is not a practical alternative to drilling mainly because of its high cost and the difficulty in use, as it needs special instruments. Also children dislike its chlorine taste and odor [2].

Later on, a new formula was commercially available in 2003 as "Papacarie". It basically consists of papain, which is a proteolytic enzyme similar to the human pepsin as well as chloramine, which together are responsible for the Papacarie's bactericide, bacteriostatic and anti-inflammatory characteristics [4-6]. Papacarie has been found to be effective in removing infected carious dentin, easy to manipulate, simple, cheap and is more comfortable to the child dental patient than the drilling method [6-8].

After the carious tissue removal, the characteristics of the dental substrate are fundamental to the adhesion of the material and they should suit the restorative system chosen, so as not to hinder adhesive procedures that will restore the shape and function of the lost structure [9].

In the operative treatment of dentinal carious lesions, the surface resulting after cavity preparation exerts an important function in the adhesion of restorative materials [10]. Any method used for caries removal will yield a different pattern of dentin substrate. Thus, a great variety of studies can be found in the literature evaluating the residual tooth structure following caries removal using either mechanical or chemomechanical methods [11-16].

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A stable adhesion between composite resin and dental structure is fundamental to the clinical success of restorations because an adhesion failure allows the infiltration of bacteria and oral fluids that may lead to the development of a secondary carious lesion [17]. Different factors have been found to influence the quality of adhesion between the dentinal surface and the restoration. These included the presence of a smear layer produced by the process of excavation and the hybrid layer resulting from the interaction between the adhesive resin systems and the etched surface [18].

Corrêa *et al* [9] and Arora *et al*, [19] analyzed the residual dentinal surfaces by scanning electron microscopy (SEM) following caries removal using Papacarie and rotatory instruments. Papacarie showed minimal smear layer and open dentinal tubules, while rotary instruments showed a typical smear layer and exposed and occluded dentinal tubules.

From the review of literature, the initial researches of the Papacarie gel revealed promising results. Little information is available regarding the nature of dentin surface after caries removal by the Papacarie.

The aim of this work was to study the topographic features of dentin after caries removal with a chemomechanical agent (Papacarie) compared with the conventional drilling method using SEM.

MATERIALS AND METHOD

The study sample included seven exfoliated and freshly extracted primary teeth due to orthodontic reasons, with open carious dentin lesions, not reaching the pulp. Teeth were collected from out-patients attending the Pediatric Dentistry Clinic, Faculty of Dentistry, Alexandria University.

The selected teeth were thoroughly cleaned from blood and extrinsic deposits using hand scaler and fluoride free pumice. Remaining roots were removed and each tooth was sectioned with a diamond disc longitudinally through the center of the carious lesions into two halves. Samples were then divided into two groups according to the method of caries removal. *Group I:* consisted of seven halves, in which caries was removed using the chemomechanical agent (Papacarie), and acted as the test group. *Group II:* consisted of the other corresponding seven halves, in which caries was removed using the conventional drilling method and acted as the control group. Teeth were mounted in acrylic resin blocks. The self-curing acrylic resin was mixed and poured into a metal ring. Each specimen was then embedded into the resin block horizontally so that the cut tooth surface was facing upwards and uncovered by the resin. Caries removal was performed using either of the two methods:

Group I: Using the chemomechanical method 6

The Papacarie (Formula and Acao (F and A), Laboratorio Famaceutico Ltda Me. 04106001. Sao Paulo (SP)–Brazil) was applied on the carious lesion for 60 seconds. The softened decayed dentin was scraped away in a pendulum motion with a blunt excavator (Martin. Gebruder Martin. Ludwig Staler Strabe 132. Postfach 60 D-7200 Tuttlingen. Germany). The gel was reapplied whenever it appeared cloudy. According to the manufacturer's instructions, there was no need for rinsing or drying the cavity between gel applications. The procedure was repeated until the gel appeared clear and reached an unchanged light color. Complete caries removal was assessed by the visual and tactile criteria according to Ericson [20]. A cotton-pellet soaked in water was used to remove the remaining gel.

Group II: Using conventional drilling method

Caries was removed using a high speed hand piece under a cooling system with a #330 carbide bur. The cavity outline followed the guidelines of modified cavity preparation for resin restorations with the final external outline determined by the extent of caries [21]. The cavities were checked for remaining caries using the same criteria used for the chemomechanical caries removal method.

After caries removal by either method, the 14 specimens were removed from the acrylic blocks, then kept in saline to be ready for histological preparation. Samples were dehydrated by passing through ascending series of ethyl alcohol of serial concentrations 30%, 50%, 70%, and 90% for 10 minutes each, then in 100% absolute alcohol for one hour. Samples were passed in acetone for 30 minutes for neutralization of ethyl alcohol and for complete dehydration. After that, the samples were put in amyl acetate for 30 minutes for absorption of acetone and complete dehydration. The samples were kept in a Vacuum Desiccator containing silica gel to avoid rehydration. The specimens were then mounted on a copper stub using a silver paint. They were put in the glass-bell jar of the Jeol coating apparatus for 3 minutes to be covered with a thin layer of gold palladium at 200 A^0 (A^0 = Angstrom = $1x10^{-6}$ mm) which is enough for the scanning procedure without affecting the surface details of the samples. Then the samples were put in the chamber of the Jeol SEM (JSM-5300 Scanning Microscope, Jeol-Japan) operating at 15 KV to be scanned and photographed. Samples were blindly examined by the oral histologist using the SEM at different magnifications. The surface topography and the cut section of the dentin were evaluated for the followings; presence or absence of caries, smear layer and bacteria, the general features of the dentin surface, patency of dentinal tubules, the orientation of collagen fibers, and the condition of peritubular and intertubular dentin.

The Ethical Committee at the Faculty of Dentistry, Alexandria University approved the research protocol.

RESULTS

Examination of the dentinal surfaces following caries removal by the chemomechanical agent (Papacarie) and the conventional drilling method, using SEM, showed the following:

Group I (chemomechanical agent)

The morphological aspect of the dentinal surface after carious tissue removal using Papacarie showed an irregular, porous, rough and globular appearance (Figure 1). The dentin surfaces were generally free of smear layer and the

Figure 1. Scanning electron micrograph of dentin surface in group I (the test group) showing: a) irregular, rough and porous surface (x1500); b) higher magnification showing globular protrusions around obvious dentinal tubules openings (x3500).

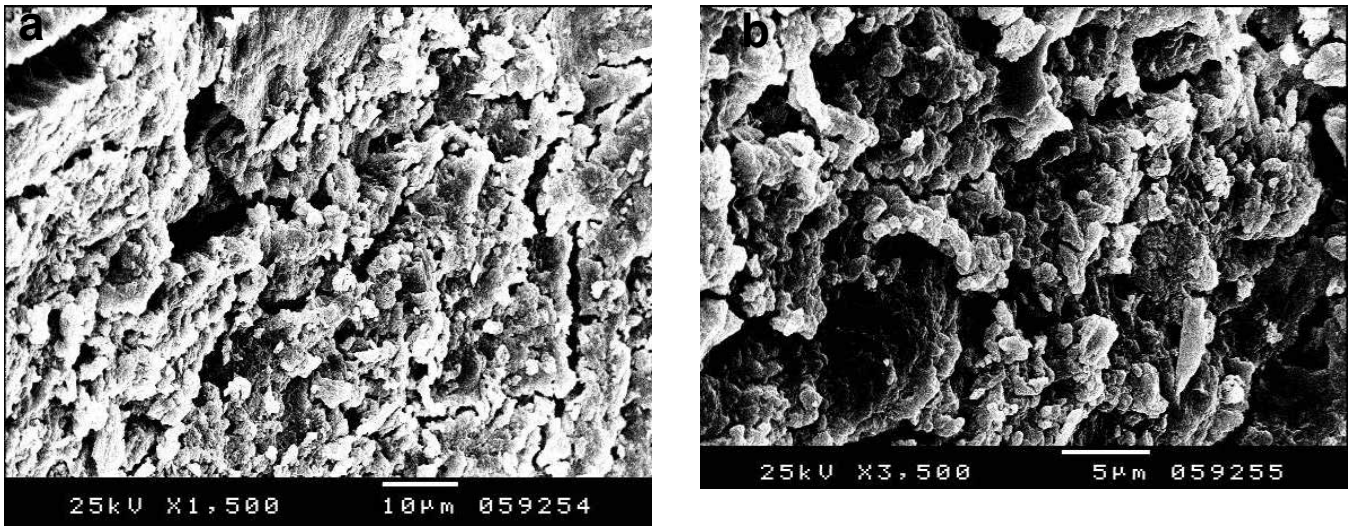
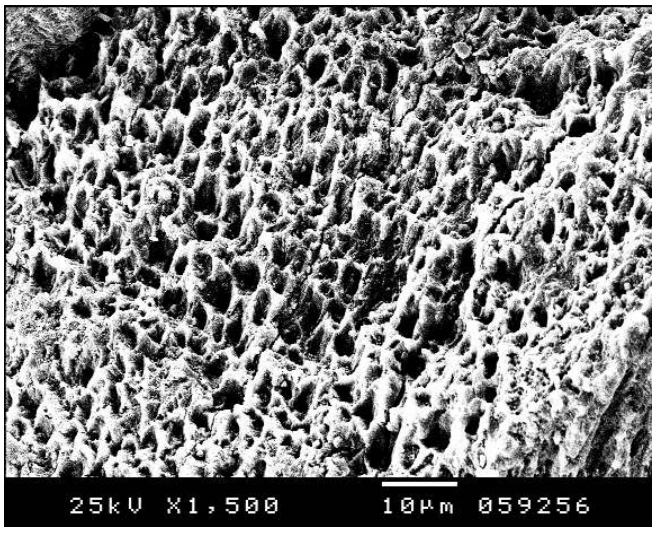


Figure 2. Scanning electron micrograph of dentin surface in group I (the test group) showing absence of smear layer with opened dentinal tubules (x1500).



dentinal tubules were opened (Figure 2). However, in some areas residual smear layer was seen occluding the orifices of the dentinal tubules (Figure 3a). At a higher magnification, some dentinal tubule openings were seen through this layer (Figure 3b). No clearly visible bacteria could be detected on the dentin surface (Figure 4).

Scanning electron micrographs of the dentin cut surfaces showed patent dentinal tubules with open orifices and irregularities in the wall and floor of the cavity surface (Figures 5, 6). The cut dentinal wall also showed intact peritubular dentin and some porosities in the intertubular dentin. The exposed bundles of the collagen fibers appeared to be disoriented (Figure 7).

Figure 3. Scanning electron micrograph of dentin surface in group I (the test group) showing: a) residual smear layer occluding many orifices of the dentinal tubules (x1500); b) higher magnification showing few opened dentinal tubules (x3500).

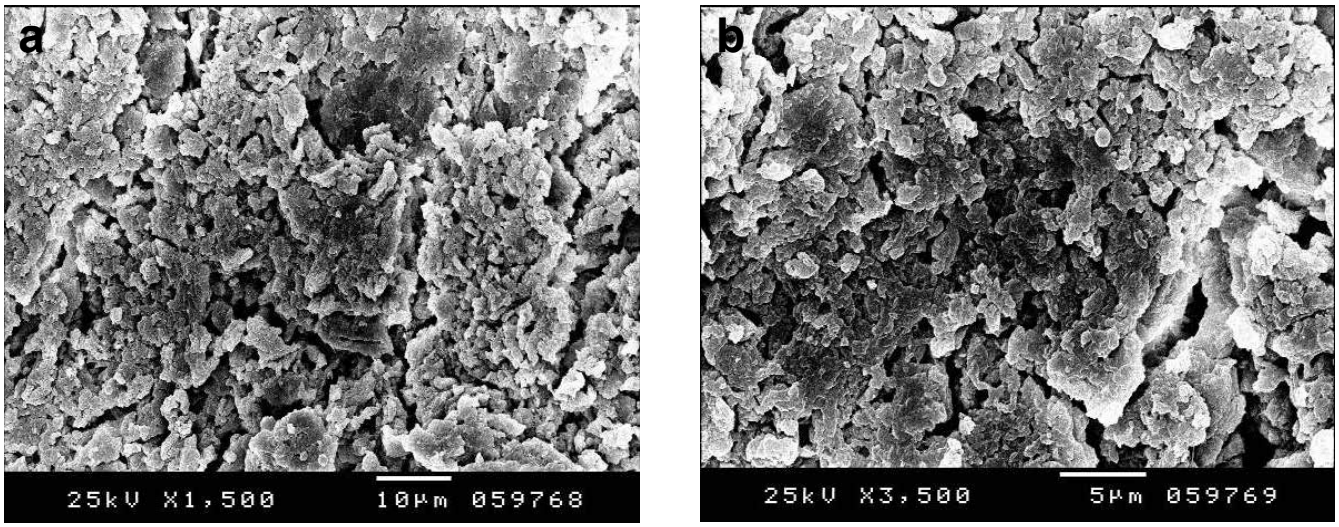


Figure 4. Scanning electron micrograph of dentin surface in group I (the test group) at high magnification showing dentinal tubule openings with no visible bacteria on the dentin surface (x7500).

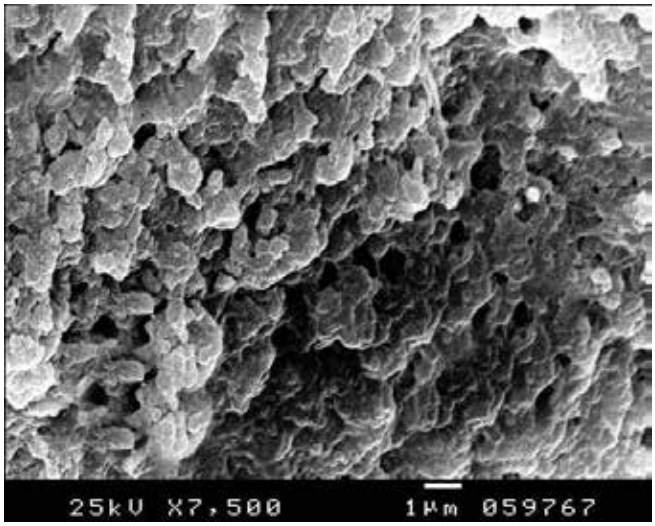
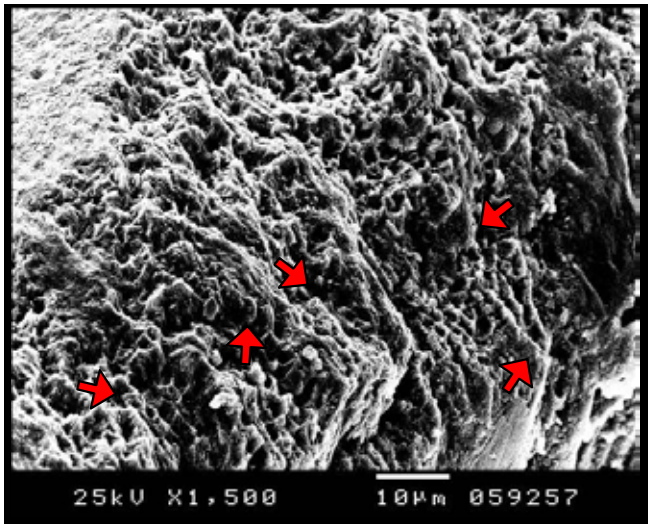


Figure 5. Scanning electron micrograph of cut dentinal section in group I (the test group) showing irregularities of the surface. Exposed collagen fibers can be noticed in some areas (red arrows) (x1500).

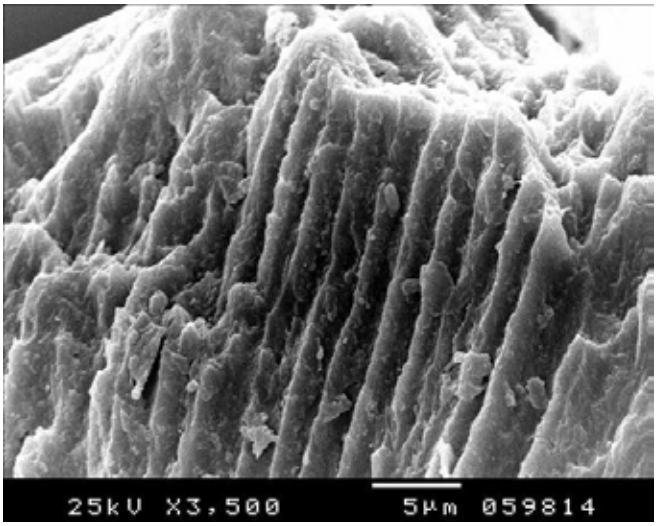


Group II (conventional drilling method)

The morphological aspect of the dentinal surface after carious tissue removal using the traditional drilling method was also examined by scanning electron microscope. It showed a smooth and amorphous surface with a typical continuous smear layer occluding the dentinal tubules. The orifices of the dentinal tubules were not observed (Figures 8, 9). Numerous bacteria were clearly visible on the dentinal surface (Figure 10).

Scanning electron micrographs of the cut dentin surfaces showed patent dentinal tubules with their orifices plugged with smear layer (Figure 11a). Higher magnification showed that the smear layer partially penetrated the dentinal tubules (Figure 11b). It also showed intact peritubular and intertubular dentin (Figure 12).

Figure 6. Scanning electron micrograph of cut dentinal section in group I (the test group) showing irregularities of the surface and opened orifices of patent dentinal tubules (x3500).



DISCUSSION

The SEM study was done to evaluate the morphological characteristics of the dentin surface following the CMCR and the conventional methods. As natural carious lesions were used, it was not possible to standardize all variables of the sample. Variables such as age, size, depth and activity status of the lesions as well as presence of tertiary and sclerotic dentin may contribute to variation in the dentin surface morphology [22-24]. Therefore, a split-tooth design was used to minimize these variables allowing for comparison between different caries removal methods.

The results of this study revealed distinct differences in the dentin surface as well as the cut longitudinal section between the two groups regarding surface texture and morphology. In group I, where caries was removed using Papacarie, no infected dentin was observed which might confirm the efficiency of the gel in removing caries as reported from previous studies [6, 7]. The Papacarie proteolytic effect is enhanced by the disrupting effect of chloramines on degenerated collagen of carious dentin and thus facilitating its removal [6, 25].

The dentin surface demonstrated an irregular, porous and globular appearance. The irregularities might be produced following the scraping away of the softened decayed tissue leaving behind the exposed affected dentin and protruded dentinal tubules. Generally, the surface showed opened dentinal tubules with absence of smear layer. This was accompanied with areas of residual smear layer occluding the dentinal tubules. According to Eick *et al* [26], the smear layer is an amorphous layer of organic and inorganic debris which is formed on the dentin surface after accomplishment of cavity preparation and removal of the carious tissue. It adheres firmly to the dentin surface from where it cannot be removed by the ordinary water spray and prevents resin from adhering to dentin.

A possible explanation of the absence of the smear layer is the presence of chloramines as one of the components of

Figure 7. Scanning electron micrograph of cut dentinal wall in group I (the test group) showing: a) opened orifices of dentinal tubules (x1500); b) normal peritubular dentin with microporosities in the intertubular dentin (yellow arrows). Note the disoriented exposed collagen fibers (red arrows) (x3500).

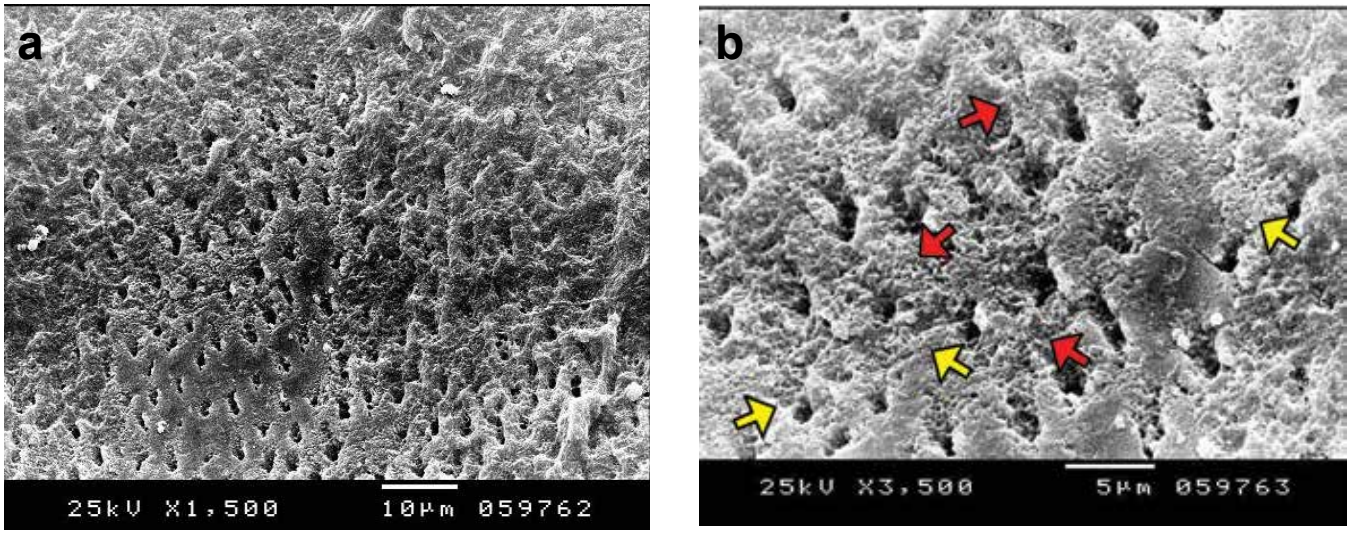


Figure 8. Scanning electron micrograph of dentin surface in group II (the control group) showing: a) dentin surface covered by a continuous smear layer in the form of linear irregularities (x1500); b) higher magnification showing the smear layer obscuring all dentinal anatomy (x3500).

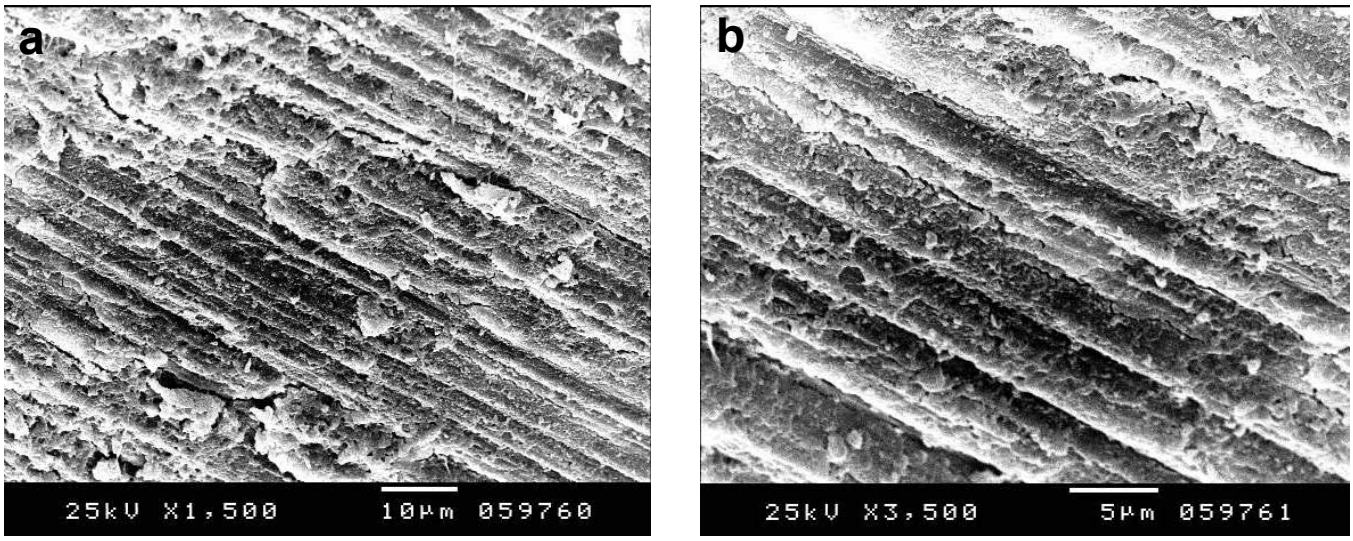


Figure 9. Scanning electron micrograph of dentin surface in group II (the control group) showing an amorphous dentin surface with smear layer occluding the dentinal tubules.

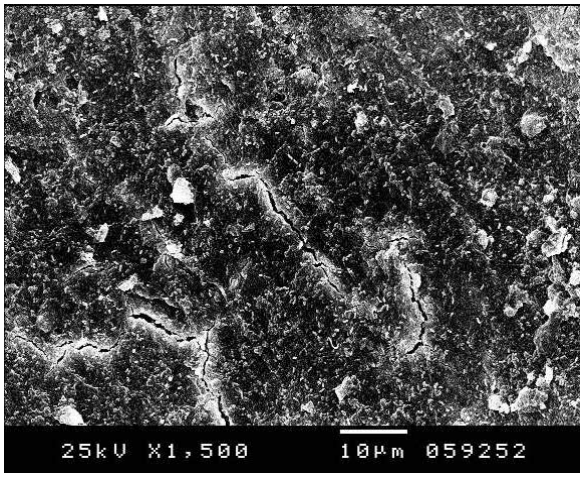


Figure 10. Scanning electron micrograph of dentin surface in group II (the control group) with visible bacteria on the dentin surface (x7500).

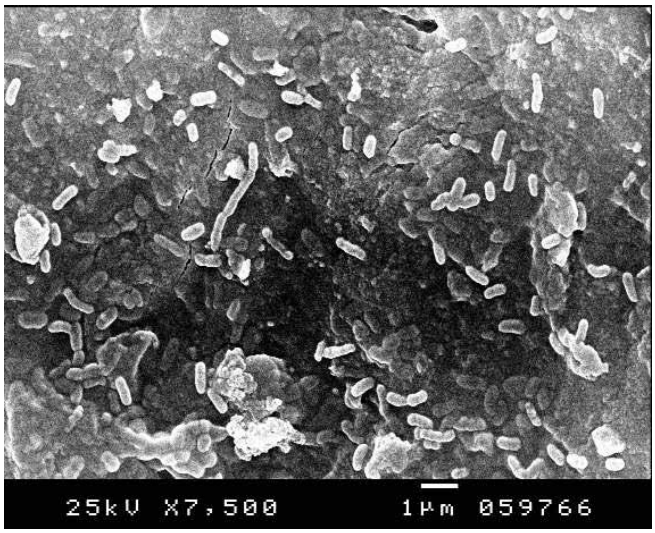


Figure 11. Scanning electron micrograph of the cut dentin section in group II (the control group) showing: a) patent dentinal tubules occluded by dense smear layer (x1500); b) higher magnification showing partial penetration of the smear layer in the dentinal tubules (x3500).

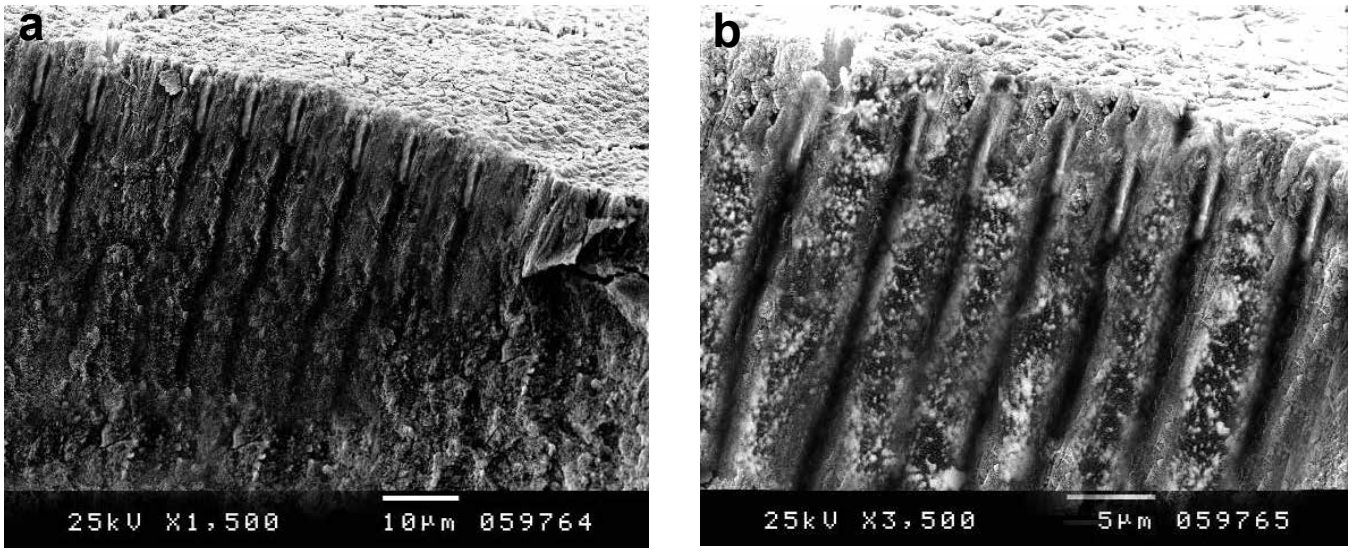
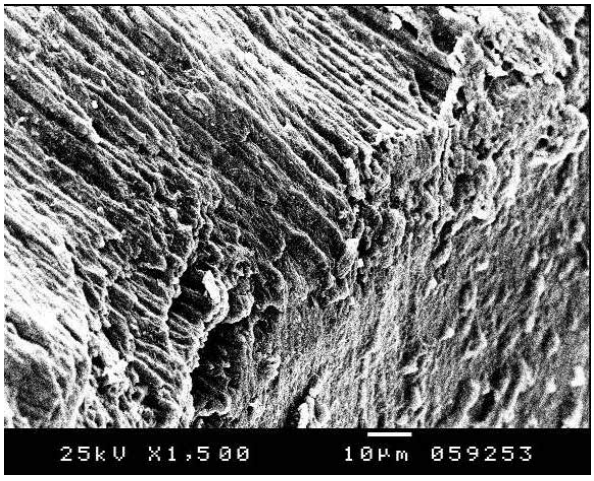


Figure 12. Scanning electron micrograph of the cut dentin section in group II (the control group) showing patent dentinal tubules with intact peritubular and intertubular dentin (x1500).



the Papacarie. According to Tonami *et al* [14], chloramines tend to chlorinate and dissolve the denatured organic components leaving opened dentinal tubules. However, the presence of some areas occluded with smear plugs may be attributed to residual debris remaining overlaid on the tubule openings following the gentle excavation to remove the softened and decomposed carious tissue. Another possible explanation is the difficult accessibility for excavation in these areas due to the small size of the specimen that was confined within the resin block.

Studies have showed that the smear layer formed after treatment with Papacarie may appear irregular and with open tubules or with minimal formation of smear layer in both primary and permanent teeth [9, 19]. Corrêa *et al*, [9] studied the residual dentinal surfaces after caries removal with Papacarie by SEM. They stated that the surfaces treated with Papacarie exhibited two different patterns of remaining dentin. A regular surface with little smear layer and exposed dentinal tubules and a very irregular and rough surface covered by an amorphic layer indicating the presence of smear layer obliterating the dentinal tubules. Similar results were obtained by Arora *et al* [19].

The results of the present study concurred with that obtained with the Carisolv in several researches. Banerjee *et al* [27] reported that Carisolv excavation consistently removed the smear layer leaving open dentinal tubules, and the surface was flaky and roughened. Similar results were obtained by Elkashlan [28], Cardoso [29] and Hahn *et al* [30]. On the contrary, Yazici *et al* [13] and Cederlundl [31] found that Carisolv failed to remove the smear layer and no patent dentinal tubules were visible.

The cut section in group I specimens revealed intact peritubular dentin with some porosities and disoriented exposed collagen fibers in the intertubular dentin. This might imply the presence of an affected demineralized dentin layer. According to Fusayama [32], the peritubular and intertubular apatite crystals of caries-affected dentin are smaller in size and less numerous than sound dentin. Microhardness studies showed that the dentinal floors of cavities after Carisolv and Papacarie treatment presented lower microhardness, suggesting that they are less mineralized than the underlying sound dentin [33, 34].

Concerning the organic matrix in the affected dentin, research findings are controversial. Sakoolnamarka *et al* [12] and Kuboki *et al* [35] found that the affected dentin showed an irregular orientation of the exposed collagen fibers compared to sound dentin. They also considered this change to be reversible under favorable condition. This result is consistent with the finding of the present study. On the contrary, Ohgushi and Fusayama [36] and Nakornchai *et al* [37] showed that the affected dentin had dense, regularly arranged collagen fibers with characteristic cross-bands similar to those in the sound dentin.

On the other hand, the dentin surface in group II showed a smooth and amorphous surface with a typical continuous smear layer occluding the dentinal tubules and obscuring all dentinal anatomy. The cut section showed patent dentinal tubules with their orifices plugged and partially penetrated with smear layer. This feature might be related to the drilling action upon dentin causing compaction and spreading of the dentin chips over the moist cavity surface. These results are in agreement with Sakoolnamarka *et al* [12] and Banerjee *et al* [23] who reported that the traditionally prepared surface appeared smooth and featureless because of the presence of a dense smear layer occluding the dentinal tubules.

The present topographic study also showed normal peritubular and intertubular dentin. This probably indicates that the drill removes both the infected and affected dentin reaching to the underlying sound dentin, while the Papacarie removes only the infected dentin and preserves the affected dentin.

The scanning electron microscopic observations revealed the presence of bacteria on the dentin surface left after the use of drill in group II, while in group I, no bacteria were visible. This finding might be due to the bactericidal and bacteriostatic properties of papain [38-40] and chloramines [41], which may affect the number of microorganisms found in the dentin following the removal of carious tissue [42-44].

The results of the present study concerning the bacterial absence are not considered confirmatory, because the histological study cannot differentiate between viable and dead bacteria [45]. It is noteworthy that they imply another possible advantage of the Papacarie over the traditional drilling as no clearly visible bacteria was detected with Papacarie.

Since the principles of minimal intervention approach indicates the need to remove only dental tissue to the extent that is strictly necessary for treatment [46, 47]. Therefore, the development of a self-limiting caries removal technique would be of great clinical importance. It appears from the results of the present study that the utilization of Papacarie as a mean for caries removal in opened dentinal lesions has presented encouraging outcomes. However, further studies on Papacarie validity in removing infected dentin, while preserving the affected one are recommended.

CONCLUSIONS

The topographic study showed that Papacarie produced a rough and porous surface with partial or complete removal of the smear layer and opened dentinal tubules, while the drill produced smooth surface completely covered by smear layer occluding the dentinal tubules.

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