Effect of antimicrobial agents on the micromorphology of primary dentin

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The use of disinfectants on the dentin surface contributes to superficial cleaning and removal of remaining microorganisms of the carious process. However, the effect of these agents on the adhesion of resin materials to the dentin surface of primary teeth has not been established. This study evaluated the dentin micromorphological effects due to the use of antimicrobial agents in the different depths of dentin. Twenty-five buccal surfaces of primary molars were prepared from the sectioned teeth, ground flat and polished with silicon carbide paper of different grits. The surfaces were treated with 32% phosphoric acid with benzalkonium chloride (Bisco), 35% phosphoric acid gel (3M), 3% benzalkonium chloride solution by itself or 10% maleic acid. All agents were applied for 15 seconds on the dentin surface. The samples were washed with distilled water, dried and coated with gold for observation of the dentin surface with the scanning electron microscope. The samples were subdivided according to the dentin depth in upper, middle and lower areas. The following was observed: presence of smear layer, opening of the dentin tubules, and inter and intratubular dentin morphology. The effects of the antimicrobial agents were compared in the different depths of the dentin. The results demonstrated that the surface conditioned with 3% benzalkonium chloride solution presented few disclosed dentin tubules. The use of the 32% phosphoric acid with benzalkonium chloride completely removed the smear layer and increased the diameter of the dentin tubule openings. The 35% phosphoric acid totally exposed the tubules in the upper area of the analyzed surface but partially exposed them in the middle and lower areas. The 10% maleic acid totally removed the smear layer, left all the tubules opened and conditioned the intertubular dentin. It was concluded that in the different depths of the dentin surface, conditioning with 10% maleic acid in primary teeth showed the most consistent micromorphological characteristics compared to the other agents used in this study.
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INTRODUCTION

Resin hybridization consists of the diffusion of resin monomers into the conditioned dentin, increasing the bond strength of composites. This process depends on the substrate quality and the amount of mineral removed during acid etching.[1,2] It has been suggested that when treating primary teeth, the use of the same types of acids, followed by the same conditioning time as for permanent teeth should be used. These suggestions do not take into account the morphological and functional differences presented by the primary teeth.[3]

Johnsen[3] highlighted some important differences between primary and permanent teeth. Primary teeth have a lesser mineralization degree and hardness than permanent teeth. Besides, the peri- and intertubular dentin present a smaller amount of phosphorus and calcium.[3] However, the mineral composition and micromorphology of the primary teeth still need further evaluation.

It is known that both the removal of the dentin smear layer and the adhesion process are directly

dependent on the quality of the substrate. Certain characteristics of primary teeth may contribute to decrease the effectiveness of the adhesives, as demonstrated by the low shear bond strength values for primary teeth compared to permanent ones.

Despite the development of adhesives that allow dentin sealing, one of the main problems with restorative materials is microleakage. Microleakage is defined as the passage of bacteria, fluid, chemical substances and ions between the dental wall and the restorative material.[9]

Despite the attempt to seal the margins of the restoration, debris of infected dentin may remain after using rotating instruments. It is important to remove carious tissue, while doing cavity preparations as the remaining bacteria may produce irritation and inflammation of the pulp tissue. This results from the bacterial proliferation in the residual layer, that allow bacterial toxins to spread into the pulp.[10, 11] Also, microleakage can occur and theoretically the bacterial proliferation can become viable starting from the nanoleakage within the hybrid layer formed.[12, 13]

Nevertheless, cleaning the cavity may be accomplished after the removal of the carious tissue and cavity preparation of the dentin, because the removal of the carious tissue alone does not guarantee the antiseptic condition even when dyes are used during cavity preparation.[14-20] Perdigao *et a1*.[15] observed that the use of chlorhexidine as a cavity disinfectant after conditioning of the dentin did not decrease the resistance of the adhesion of the All-Bond 2 adhesive system, corroborating the results obtained by Filler *et al.*[16] in enamel.

Although disinfectants based on iodine potassium and chlorhexidine do not remove the smear layer; they can modify its appearance and permeability, by removing residues. They are also effective reducing the levels of *S. mutans*.[17, 18] In addition, these products did not reduce the potential of residual caries and postoperative sensitivity when they were used after the cavity preparation and before the application of the adhesive system.[19]

Perdigao *et al.*[15] showed that the use of the 2% chlorhexidine before the application of the adhesive systems Syntac or Tenure did not affect the sealing capacity of those materials in permanent teeth. They reported that the use of 2% chlorhexidine contributed to decrease the microleakage, which was attributed to the modifications produced on the smear layer as this disinfectant cannot remove the smear layer. On the other hand, Tulunoglu *et al.*[20] reported that chlorhexidine did not interact with the adhesive systems Syntac and Excel & Bond, and significantly increased the microleakage, raising the question on the use of disinfectants prior to adhesive restorative procedures.

Benzalkonium chloride is a detergent and a disinfectant used in infection control in surgical procedures. It facilitates the cleaning and decreases surface tensions,[21] and has been marketed as a dentin etching agent in combination with a 32% phosphoric acid gel (UniEtch, Bisco Inc, Schaumburg, IL, USA).

Disinfectants and detergents are used to eliminate bacterial contamination in the permanent dentin; however, there are morphological and functional differences between the dentin of primary and permanent teeth. This study was done to analyze the effect of the use of a phosphoric acid etchant containing benzalkonium chloride, on the micromorphology of the primary dentin.

MATERIALS AND METHODS

Twenty-five primary teeth extracted for orthodontic reasons from children aged 6 to 9 were used. Immediately after the extraction the teeth were stored in a 2% glutaraldehyde solution for 2 to 4 hours. Next, they were washed and stored in a saline solution. The sound buccal surfaces were cleaned with water slurry of pumice with a soft rubber prophylaxis cup rotating at low speed; then stored in saline solution at room temperature. The roots of the teeth were removed and each crown was horizontally mounted in phenolic rings and self-cured acrylic resin with the buccal surface exposed.

The specimens were then randomly assigned to five groups:

Group 1. Untreated and control group;

Group 2. Etched with 32% phosphoric acid with benzalkonium chloride (UniEtch, Bisco Inc., Schaumburg, IL - Lot -029185), for 15 seconds;

Group 3. Etched with 35% phosphoric acid (Scotchbond Etching Gel, 3M Dental Products, St. Paul, MN, Lot 3AE), for 15 seconds;

Group 4. Etched with 3% benzalkonium chloride in alcohol solution;

Group 5. Etched with 10% maleic acid (3M, St. Paul, MN).

In all specimens, the buccal surface was ground wet in a polishing machine (PF Dujardin & Co, Dusseldorf, Germany) with 320-600 grit silicon carbide papers to create a flat dentin surface. Specimens were treated according to each group described above. After etching, the specimens were washed in distilled water for 15 seconds and critically point dried (CPD 020, Balzers Union). Next, they were submitted to eight changes of liquid CO_2 and covered with a 10 mm thick layer of gold (MED 010, Balzers Union) and observed in a scanning electron microscope (DSM 940A, Carl Zeiss, Munich, Germany) operating at 5-10 KV, with x1500 magnification. The three distinct regions, upper, middle and lower, were analyzed.

Group 1, which was the control group, had a homogeneous smear layer covering the dentin surface and all dentin tubules (Figure 1).

Group 2, which was treated with the 32% phosphoric acid with benzalkonium chloride, showed a total disclosure of the dentin tubules and this was observed

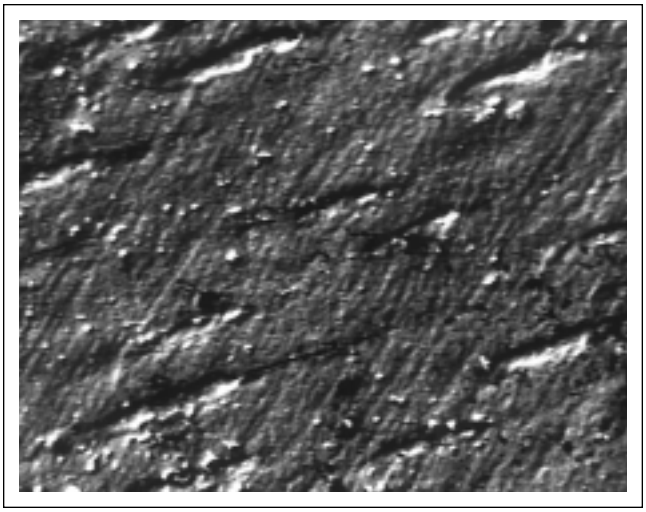

Figure 1. Untreated specimen (control group). Note the presence of smear layer on the dentin surface with no evidence of exposed dentin tubules (x l500).

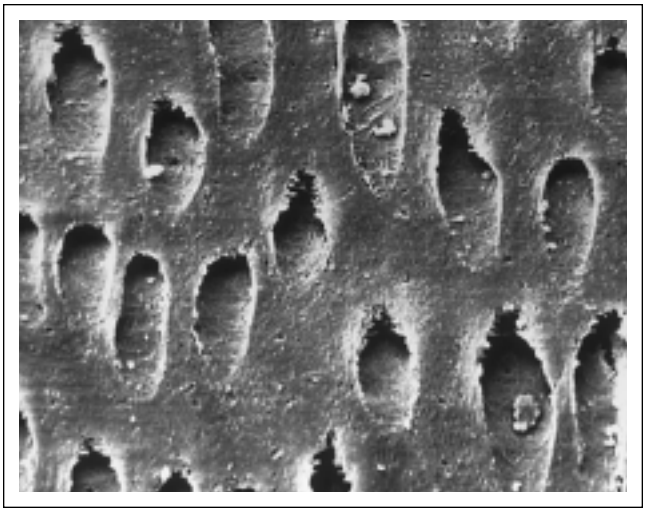

Figure 2. Specimen etched for 15 seconds with a 32% phosphoric acid semigel (UniEtch, Bisco) containing benzalkonium chloride. Dentin tubules are totally exposed. Intertubular dentin etching was less effective compared to the 10% maleic acid. (x l500).

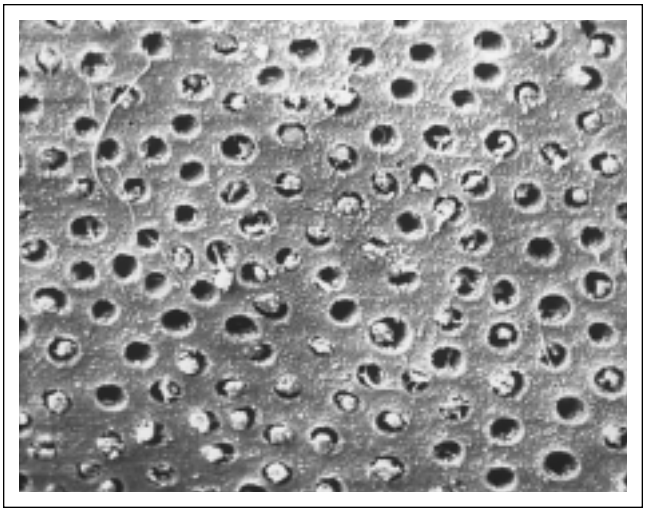

Figure 3. Specimen etched with 35% phosphoric acid (Scotchbond Etching Gel, 3M) for 15 seconds. Dentin tubules are partially covered by smear layer. The intertubular dentin is slightly conditioned. (x l500).

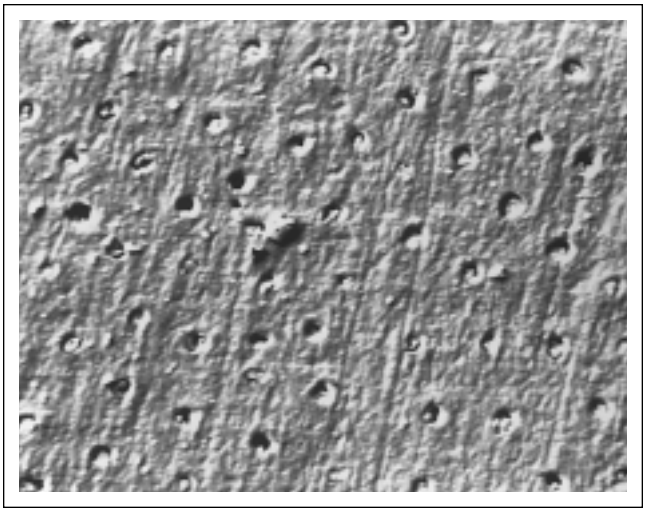

Figure 4. Specimen treated with 3% benzalkonium chloride in an alcoholic solution. The dentin tubules are partially covered by the smear layer. The intertubular dentin was not conditioned. (x l500).

in all the analyzed areas (Figure 2). The intertubular dentin was also conditioned. An increase in the diameter of the opening of the tubules was greater than that observed in the conditioning with the 10% maleic acid specimens (Group 5).

Group 3 was treated with 35% phosphoric acid to etch the dentinal surface. The dentinal tubules on the upper area were totally opened and in the medium and lower areas partially opened. The intertubular dentin was less etched (Figure 3). The diameter of the opening of the dentin tubules was smaller than the samples of Groups 2 and 5.

Group 4 was treated by using a conditioning agent with 3% benzalkonium. It was found that most of the dentin tubules were partially obstructed. The entire surface of the intertubular dentin was not conditioned (Figure 4).

Group 5 dentin was conditioned with 10% maleic acid. The dentin tubules were totally opened in all the evaluated areas. The intertubular dentin was well conditioned, as evidenced by the irregularities present on the surface (Figure 5).

In all groups, the images of the lower third area of the dentin showed a larger number of tubules with smaller diameters when compared to the middle third area.

When the dentin was conditioned with 10% maleic acid, collagen fibers were observed (Figure 6).

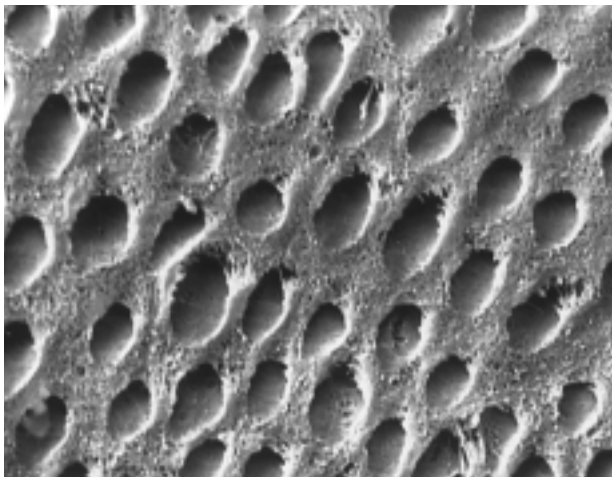

Figure 5. Specimen conditioned with 10% maleic acid. The dentin tubules are totally disclosed. This conditioning agent was the most effective to etch the intertubular dentin. (x l500).

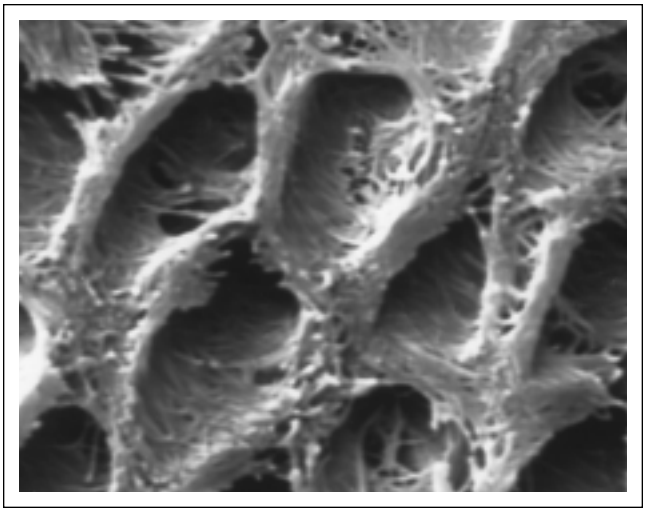

Figure 6. With 10% maleic acid conditioning the collagen network was readily exposed. (x 1500).

DISCUSSION

Conditioning with 3% benzalkonium chloride (Group 4) did not show a satisfactory performance, as most of the dentin tubules remained obstructed by the smear layer. Consequently, it did not etch the intertubular dentin. Although benzalkonium chloride can be classified as a disinfectant and a detergent, it did not show a satisfactory detergent action on the dentin. In a recent study, Puppin Rontani *et al.*[22] observed that there was no hybrid layer formation when 2% and 3% benzalkonium chloride solutions alone were used, suggesting the non-removal of the smear layer.

The use of the 35% phosphoric acid (Group 3) on the dentin surface was shown to be more effective to remove the smear layer. The corresponding area of the upper third of the dentin showed the dentin tubules totally opened, and in the middle and lower thirds they were partially opened. In this group, the intertubular dentin clearly showed signs of conditioning, as observed by the presence of irregularities on its surface. Settembrini *et al.*[23] verified that acid etching of the dentin removed the smear layer, opened and enlarged the dentin tubules. They observed low microleakage in the dentin margins and significant antimicrobial properties that could be beneficial against the remaining caries.

When conditioning was accomplished with the 32% phosphoric acid containing benzalkonium chloride a total disclosing of the dentin tubules with the complete removal of the smear layer was noted. The intertubular dentin was conditioned in a more efficient way when compared to the use of the 35% phosphoric acid only. This conditioning promoted the clearest increase in the dentin tubules opening in relation to the other groups. Kanca[24] reported that there was no change in the shear bond strength of adhesive system to the dentin or enamel etched with phosphoric acid containing benzalkonium chloride or not.

In this study it was noted that the use of 35% phosphoric acid promoted the highest dentin surface irregularities when compared to 10% maleic acid. This may be attributed to the smaller molecular weight of the phosphoric acid, which promotes a faster decalcification because the primary teeth dentin is less mineralized and has greater permeability.[2] Maleic acid, with a larger molecular weight, and a organic acid needs more time to promote the same kind of decalcification as 35% phosphoric acid.[25] When the dentin surface was etched with 10% phosphoric acid gel for 15 or 60 seconds, it left no debris on the dentin surface because this etchant is thickened with a polymer instead of silica.[15] With 10% maleic acid gel and 15 or 60 seconds of etching time, the dentin surface revealed smear layer residues. This occurs because maleic acid is organic and has a higher molecular weight, which requires a greater time for acid reaction on a dentin surface.[26]

Although 10% maleic acid is a weak acid and used for a short period of time, in this study it exhibited an effective action by conditioning the intertubular dentin and removing the smear plugs at the entrance of the dentin tubules. This effect may suggest that maleic acid may be a better dentin conditioner for primary teeth, perhaps due the chemical characteristics and structure of the primary tooth dentin, when compared to the conditioning actions of other agents.[26, 27]

In the samples analyzed, the deeper area of the dentin showed the largest number of dentin tubules, but these were smaller in diameter when compared to those of the middle area.

Further studies should evaluate the action of disinfectants in the dentin of primary teeth, as well as any effects on the shear bond strength.

CONCLUSIONS

1. Conditioning primary teeth dentin with 3% benzalkonium chloride did not totally remove the smear layer, although few open dentin tubules were seen.
2. Conditioning primary teeth dentin with 10% maleic acid produced a total exposure of the dentin tubules and was effective in etching the intertubular dentin.
3. The 32% phosphoric acid with benzalkonium chloride also promoted total exposure of the dentin tubules and conditioning of the intertubular dentin, and created the greatest enlargement of the dentin tubule openings.
4. The deepest area of the dentin presented tubules with smaller diameter in comparison to the middle area.
5. Further studies should be conducted on the morphology of the primary teeth dentin as compared to the permanent teeth dentin to establish the morphology of these two substrates.

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