

Evaluation of Residual Dentin after Conventional and Chemomechanical Caries Removal Using SEM

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The purpose of this in vitro study was to analyze the residual dentinal surfaces following caries removal using rotatory instruments and two chemomechanical methods (Papacárie® and Carisolv®), by scanning electron microscopy (SEM). Thirty primary incisors were divided into three groups, according to the caries removal method used, and their residual dentin was examined under SEM (15). After caries removal, 15 of these teeth were restored with Single Bond (3M) adhesive system and Z100 Filtek composite resin (3M). The tags of the replicas were observed under SEM. The chemomechanical caries removal methods (Papacárie® and Carisolv®) formed an amorphous layer, similar to the smear layer and few exposed dentinal tubules; the conventional caries removal method produced a smooth and regular dentinal surface, with typical smear layer and exposed dentinal tubules. All groups showed abundant tag formation. Scanning electron microscopy analysis revealed a difference between dentin treated with rotatory instruments and that treated with chemomechanical methods in spite of the occurrence of a similar tag formation in both groups.

Keywords: chemomechanical removal; dental caries; primary teeth, scanning electron microscopy, carisolv, papacarie

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INTRODUCTION

Although the evolution of adhesive materials has led to a decrease in the amount of healthy dental tissue that is removed during cavity preparation, low and high speed rotatory instruments have long been the main instruments used by dentists who, when in doubt, used to open up the cavity or remove a great part of healthy dental structure.

The main disadvantages of the traditional treatment are: The possibility of overextending the cavity and removing healthy tissue, which may lead to pulp exposure; heating; pressure exerted on the pulp; vibration; noise; pain stimulus and the need for local anesthesia, which causes aversion in many patients, especially in children.

New methods of carious tissue removal have been developed as an alternative to conventional treatment: Laser abla-

tion, air abrasion, ultrasound, chemomechanical removal, as well as new restorative techniques like Atraumatic Restorative Treatment. Such techniques may serve as a way to win over fearful patients, enabling good behavior management, diminishing discomfort, and minimizing the need of anesthesia.

The main objective of chemomechanical caries removal is to eliminate the outermost portion (infected layer), leaving behind the affected demineralized dentin that can be remineralized and repaired.^{1,2} Countless studies have been carried out in order to evaluate the clinical efficacy and safety of Carisolv®, many of which have pointed out that the majority of patients did not report discomfort during the treatment^{1,3,4,5} and local anesthesia was rarely needed.^{6,7} Tamay *et al.* (2001) have pondered that Carisolv® is a promising material, especially for the treatment of primary teeth since it offers more comfort to the patient than the conventional method of caries removal.^{9,10} As a disadvantage, authors who have assessed this product point out that caries removal takes longer than the conventional method with burs.^{4,10,11,12,13}

In 2003, with the purpose of presenting a chemomechanical caries removal product less costly than Carisolv®, a material composed of papain, chloramine, and toluidine blue called Papacárie® was launched. Papain is an endoprotein with bacteriostatic, bactericide, and anti-inflammatory properties.¹⁴ Chloramine contains chloride and ammonia, possesses bactericidal and disinfectant properties, and it is used for root canal irrigation or chemical softening of carious

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dentin, chlorinating the degraded collagen portion of the dentin during the chemical and mechanical process of caries removal.⁷

After 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.

The dentin substrate is a hydrated biological complex with different regional characteristics that may be altered by physiological processes, age and diseases¹⁵. Significant variations in its architecture may occur according to the depth and to the response to previous aggressions such as carious lesions, cavity preparations, and possible aggressive effects of restorative materials.¹⁶

Any method used for caries removal will yield a different pattern of dentin substrate. Thus, a great variety of studies can be found in literature evaluating the remnant tooth structure after caries removal using mechanical and chemomechanical methods.^{10,17-33}

The adhesion between restorative materials and dental tissues is one of the study objects that have captured the interest of scientists lately. 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.³⁴ In the operative treatment of dentinal carious lesions, the surface resulting after cavity preparation exerts an important function in the adhesion of restorative materials.³⁵

A variety of factors that influence the quality of adhesion between dental surface and restoration, include 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.¹⁰

Different methods may be used for the removal of carious tissue. The decision to use one method or another is based on professional preference and on the indication for each individual case. Dentin adhesion depends not only on the adhesive system used but also on the dentinal substrate remaining after carious tissue removal.

Therefore, given the relevance of this subject, the emergence of new caries removal materials, and the scarcity of studies regarding this issue on primary teeth, the present research aimed at evaluating remaining dentin and tag formation in primary teeth using three different methods of carious tissue removal.

MATERIAL AND METHODS

This research protocol received previous consent from the Research Ethics Committee, School of Dentistry, University of São Paulo (protocol number 167/04).

Thirty extracted central primary incisors, with an active carious lesion in a single interproximal surface, were divided into three experimental groups as follows: Conventional mechanical treatment – low speed rotary instrument – and two chemomechanical methods - Papacárie™ and Carisolv®.

Cariou tissue removal using the conventional technique was performed with a spherical steel bur (Wilcos do Brasil, Petrópolis- Brazil) with the largest diameter compatible with the cavity size, at low speed, under cooling, by a single operator. In order to gauge carious tissue removal, an exploratory probe was used to check, until hard dentin was obtained.

For the Papacárie™ (Fórmula & Ação, São Paulo, Brazil) and Carisolv® (Medi Team, Goteborg, Sweden) groups, the product was applied and left in the cavity for 30 seconds, and carious dentin was afterwards removed with a blunt Maileffer curette (Ballaigues, Switzerland) that belonged to the Carisolv® system. The gel was reapplied until it presented a light coloring, indicative of non-existence of softened carious tissue. This was substantiated with the use of the exploratory probe, to assess the remaining dentin hardness.

Immediately after treatment, the samples were prepared for scanning electron microscopy analysis, according to Perdigão¹⁹⁹⁵.

For tags and microtags evaluation, another 15 similar primary central incisors with dentinal caries lesion were obtained from the Human Tooth Bank of the School of Dentistry of the University of São Paulo and were randomly divided into three groups of five elements according to the type of caries removal to be used. The procedures were done as aforementioned.

After obtaining dentinal surfaces treated with the three different methods, teeth were acid etched using 37% phosphoric acid (Etchant 3M ESPE) for 15 seconds followed by rinsing, drying, and application of the Single Bond adhesive system strictly following manufacturer's instructions. A composite resin (Filtek Z100, 3M ESPE) block, A4 color, approximately 5mm in height was built using 1mm thick increments that were light-cured for 20 seconds each with an Elipar curing light (3M ESPE) of approximately 500mW/cm² light intensity.

Teeth from each study group were then immersed in 18% HCl during 48 hours in order to completely remove the dentin. They were washed in an ultrasound unit for 15 minutes using distilled water³⁷ then processed following all the steps described for SEM analyses.³⁶

All specimens were kept in a vacuum chamber (Pelco-2251) in order to prevent the oxidation of the metallized surfaces for posterior morphological analysis using scanning electron microscopy (JEOL-JXA 6400).

RESULTS

Two aspects were assessed through scanning electron microscopy analysis: the dentinal substrate resulting from each method of caries removal and the observation of the replicas obtained using an adhesive system and composite resin on surfaces that had been previously submitted to the three different caries removal methods.

The morphological aspect of the dentinal substrate remaining after carious tissue removal using low speed rotary instruments can be observed on Figure 1, showing a smooth and uniform surface with a typical smear layer and where exposed dentinal tubules can be seen.

The surfaces treated with Papacárie® exhibited two different patterns of remaining dentin. On Figure 2 a regular and “cracked” surface can be observed, with little smear layer and exposed dentinal tubules in some regions. On Figure 3, chemomechanical removal using Papacárie® resulted in a very irregular and rough surface covered by an amorphous layer indicating the presence of smear layer obliterating the dentinal tubules.

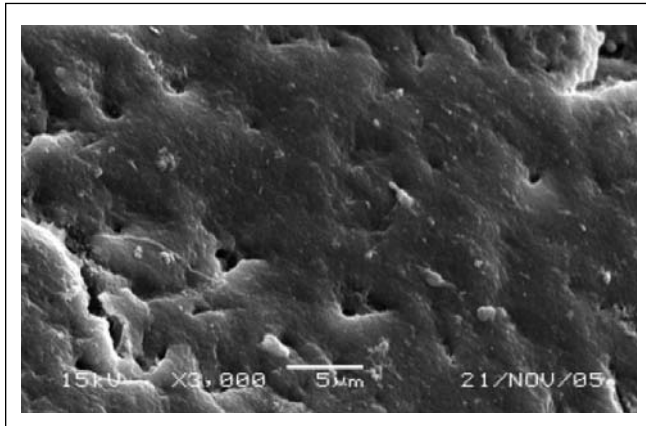


Figure 1. Dentin after removal using rotatory instruments (3000X, 5µm)

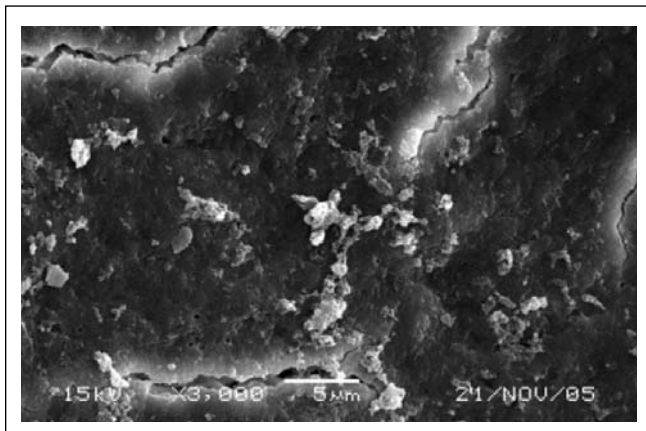


Figure 2. Dentin after chemomechanical removal (Papacárie®) (3000X, 5µm)

Scanning electron microscopy of the Carisolv® group specimens revealed an irregular surface with the presence of an amorphous layer similar to a smear layer and, in a few areas, there were exposed dentinal tubules. It can also be observed, the presence of bacterias on the dentinal surface (Figure 4).

Figure 5 represents the specimens from the rotatory instruments group showing the tooth-resin adhesive interface, where the presence of innumerable tags throughout the entire extension of the specimen can be observed. On Figure 6, the base of the tags is seen to be tapering in the tubules region and scarce microtags can be observed.

In the Papacárie® chemomechanical removal group, the formation of a great entanglement of tags occurred (Figure 7) and microtags with tapered bases could be seen as well as a few collagen fibrils infiltrated in the region where the tubules narrow down (Figure 8).

In the Carisolv® chemomechanical removal group, the formation of tags was observed throughout the whole extension of the specimen (Figure 9). On Figure 10, the tags exhibited a tapered base and, at a greater magnification, one can notice the presence of microtags at the base of the tags.

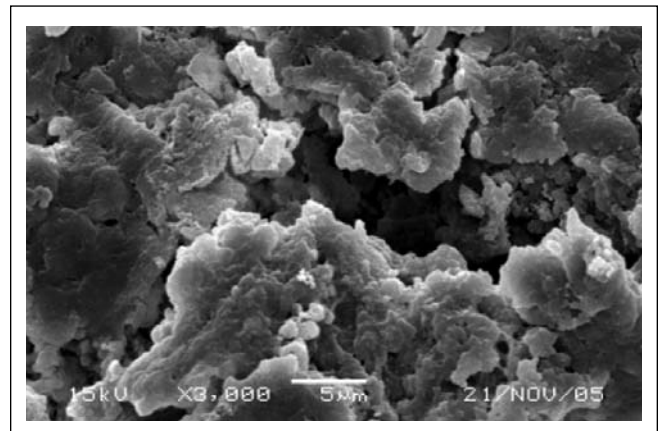


Figure 3. Dentin after chemomechanical removal (Papacárie®) (3000X, 5µm)

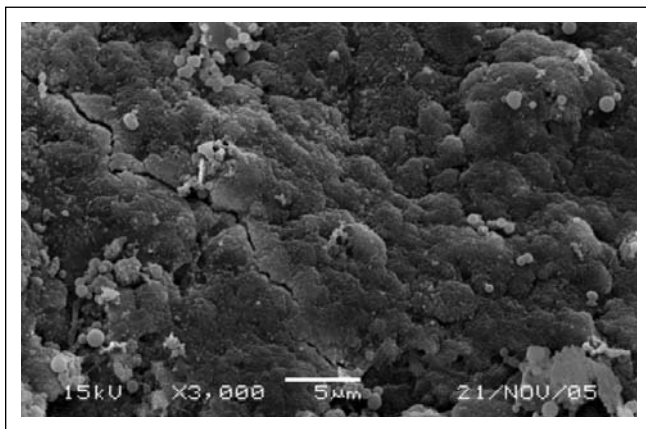


Figure 4. Dentin after chemomechanical removal (Carisolv®) (3000X, 5µm)

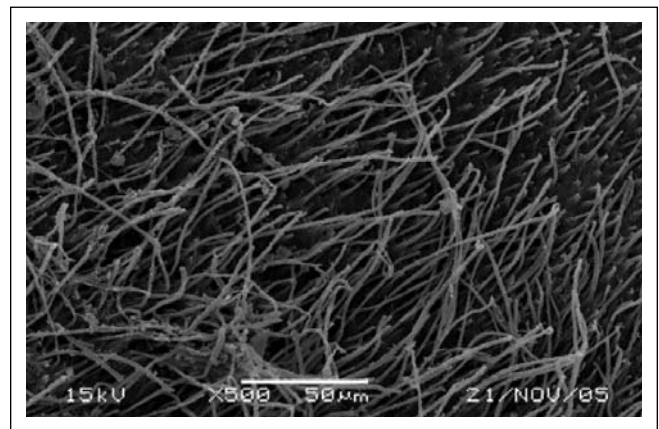


Figure 5. Electronic micrograph of a specimen from the rotatory instrument group (500X, 50µm)

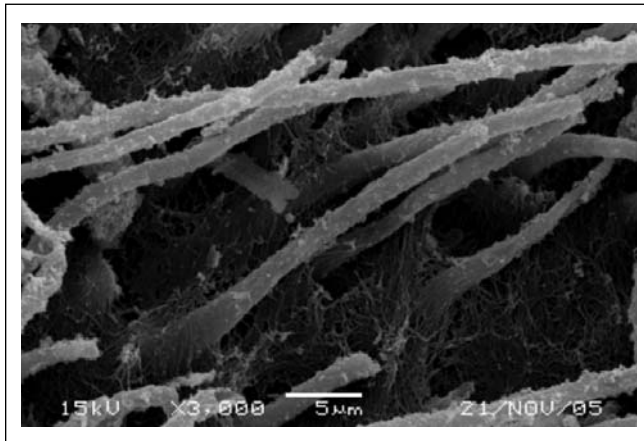


Figure 6. Electronic micrograph of the rotary instrument group. Tapered tag base and presence of collagen fibrils (3000X, 5µm).

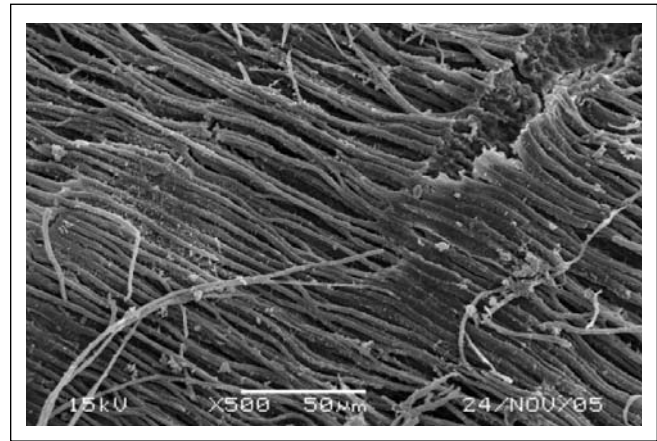


Figure 7. Electronic micrograph of a specimen from the Papacárie® group (500X, 50µm)

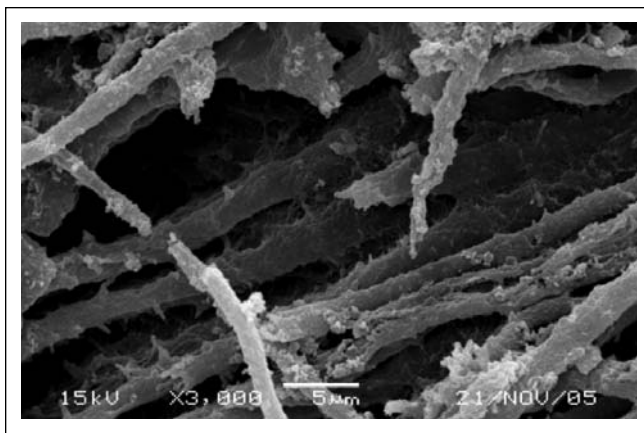


Figure 8. Electronic micrograph of a specimen from the Papacárie® group (3000X, 5µm)

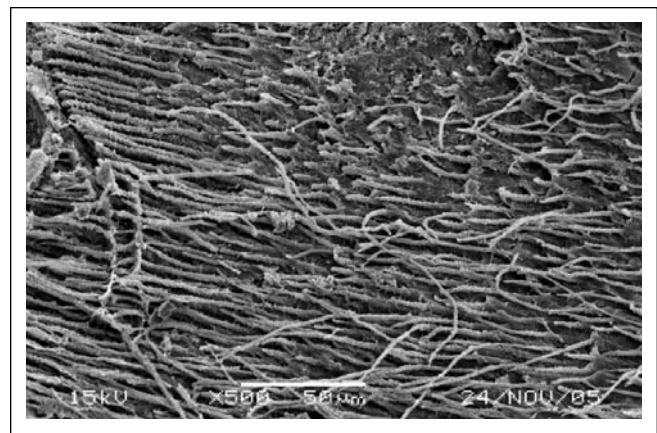


Figure 9. Electronic micrograph of a specimen from the (Carisolv®) group (500X, 50µm)

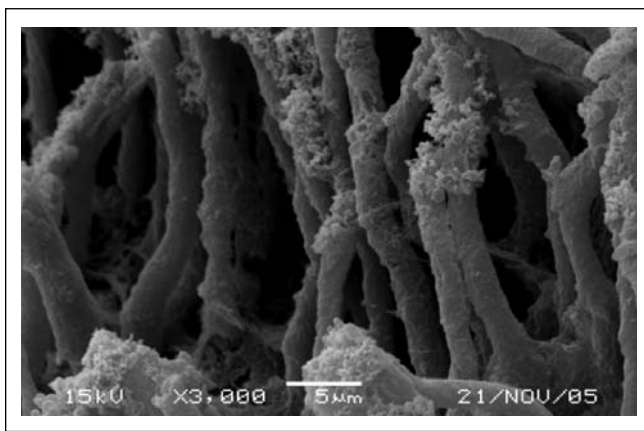


Figure 10. Electronic micrograph of a specimen from the (Carisolv®) group (3000X, 5µm)

DISCUSSION

Regarding the morphological analysis of the substrate using the micrographs obtained, it was possible to verify that different methods of carious tissue removal yielded different dentinal surfaces.

In the cutting rotary instruments group, a smooth and uniform surface was observed – with a typical smear layer –

and exposed dentinal tubules, Such observations are in accordance with various reports.^{33,17,21,22,24,25,}

In contrast, the Carisolv® group had a dentinal substrate with an irregular surface, amorphous layer, and a small number of dentinal tubules.^{21,22,24,28} Such findings were not in agreement with some authors^{10,23,28,31} who confirmed that the Carisolv® chemomechanical method of caries removal was able to remove the smear layer and expose dentinal tubules, making the dentinal surface more receptive to adhesive systems.

In the Papacárie® group micrographs, two patterns of dentinal substrates were discerned. The first pattern presented an amorphous layer similar to the smear layer and little presence of exposed dentinal tubules.³³ In another analysis, a regular surface was observed, with minimum smear layer and some regions showing exposed dentinal tubules.

The smear layer produced in dentin affected by caries possesses acid-resistant crystals that may hinder the diffusion of the primer into the intact underlying dentin. This layer acts as a barrier, decreasing the dentin’s permeability and is also considered to be an impediment to the establishment of intimate contact between tooth and resin.³⁷ On the other hand, the presence of such deposits in the dentinal

tubules may reduce the dentin's permeability, constituting a protective barrier for the pulp since it reduces the intrusion of bacteria and bacterial products.

The treatment of the dentinal surface and the resulting characteristics of the dentinal substrate will be important for adhesion and will affect the performance of composite resin restorations. All standards established for the preparation of dentinal substrates for adhesion have, so far, been studied in permanent teeth and the results were extrapolated to primary teeth without taking into consideration the differences in morphology and composition that may exist between these two types of dentitions.³⁸ It is worth noting that primary teeth dentin presents peculiarities such as a smaller number of dentinal tubules – of smaller diameter – than permanent dentin, thus making it less permeable.³⁹ Therefore, the diffusion of adhesive into the tubules may be reduced in primary teeth, leading to the formation of shorter tags.

Adhesion of resins to dentin is considered to be mainly based upon micromechanical retention. The formation of resin tags inside dentinal tubules, branching or microtags, and the formation of a hybrid layer or resin-dentin inter-diffusion zone, which is a mixture of demineralized collagen and monomers from the primer and adhesive.³⁵ Obtaining a hybrid layer involves applying an acid etch to the dentin that is able to completely remove the smear layer and smear plugs, opening up the dentinal tubules and, thus, increasing dentinal permeability as well as exposing the collagen network of intertubular dentin due to mineral removal.³⁷

Adhesion to dentinal structure is difficult due to the heterogeneous characteristics of this tissue where 70% of its composition in weight is made up of hydroxyapatite, 18% is organic material (mainly collagen) and 12% is water.⁴⁰ Moreover, dentin is a living tissue and any stimulus exerted on it reflects directly upon the pulp; its hydroxyapatite crystals are irregularly arranged in an organic matrix constituted of collagen fibers; dentinal tubules contain odontoblastic processes and dentinal fluid that extend from the pulp across to the amelodentinal junction, causing intrinsic humidity and high permeability.⁴¹

Scanning electron microscopic analysis of the resin replicas revealed innumerable elongated tags and a small amount of microtags for all experimental groups. Samples from the rotatory instrument group exhibited small fibril-like projections, indicating that mineralized collagen fibrils may have been incorporated (infiltrated) by resin monomers.³⁷

In samples from Carisolv[®] and Papacárie[®] chemomechanical removal groups, a similar tag formation occurred but no collagen fibrils were observed. This may be explained by the ability that Carisolv[®] has to degrade collagen fibers.¹⁹

Although the treatment of dentinal surfaces with chemomechanical methods has yielded a great amount of smear layer in the present study, tag formation was present in both Carisolv[®] and Papacárie[®] groups. This layer generally obliterates the entrance of dentinal tubules, reducing dentinal permeability approximately 40 times.⁴⁰

In this study, tag formation probably occurred from to the

use of acid etching removing the smear layer, allowing for the infiltration of resin adhesives. Hypochlorite found in Carisolv[®] removes the smear layer, making exposed dentin permeable, and favoring the penetration of substances such as adhesive materials and composite resins with the purpose of improving properties of retention.⁴³

The presence of tags and microtags has rarely been discussed in literature. Research has been made to assess the formation of tags according to the type of adhesive system used.^{37,44} However, there are no studies analyzing the formation of tags and microtags after using different methods of caries removal. This situation is aggravated when it comes to primary dentin due to a lack of studies regarding the specific morphological characteristics of this type of tissue. Primary dentin is different from permanent dentin in respect to thickness and number of dentinal tubules at different depths and the former has a smaller number of microcanals, thus promoting a smaller adhesion force.^{39,45}

It is important to emphasize that the development of techniques for caries prevention, as well as the improvement of restorative materials, have enabled dentists to opt for conservative tooth preparations in order to preserve dental structure. Conventional caries removal method involves the use of rotatory instruments and may be unpleasant to the patient, often making local anesthesia necessary, and at the risk of removing healthy dental tissue. Such disadvantages have led to the search for alternative methods that could guide the removal of carious tissues while also providing greater comfort to patients.

The results of the present study indicate a similarity between remaining dentinal substrates after different methods of caries removal were tested. Yet, further tests are necessary for future evaluation and comparison.

CONCLUSIONS

According to the results obtained, it is concluded that scanning electron microscopy analysis revealed a difference between dentin treated with rotatory instruments and that treated with chemomechanical methods in spite of the occurrence of a similar tag and microtag formation in both groups. The group treated with low speed rotatory showed a smooth and uniform surface with a typical smear layer and exposed dentinal tubules. The surfaces treated with Papacárie[®] 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 amorphous layer indicating the presence of smear layer obliterating the dentinal tubules. The Carisolv[®] group specimens revealed an irregular surface with the presence of an amorphous layer and bacteria on the dentinal surface.

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