# Dentine surface morphology according to caries removal method and subsequent acid etching

J. Ghilotti, P. Manzano, M. Melo, A. Lozano, C. Llena

*Universitat de València, Spain*

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Email: llena@uv.es

## Abstract

**Aim** To evaluate dentine morphology after caries removal by different mechanical and chemo-mechanical methods and subsequent orthophosphoric acid etching in permanent teeth, using a scanning electron microscope (SEM).

**Methods** Eight extracted natural teeth with caries (ICDAS score 5-6) were divided into four groups (ni=2) according to the dentine removal method. Group 1 (tungsten carbide bur, TC); Group 2 (polymer bur, PB); Group 3 (excavator, EX); Group 4 (Brix 3000®, BR). After caries removal, each tooth was sectioned mesiodistally. One half was kept in buffered saline solution while the other half was exposed to 37% orthophosphoric acid (OA) for 5 seconds. The samples were analysed under SEM conditions at different magnifications.

**Results** The groups in which mechanical systems were used showed a compact smear layer that was partially eliminated after etching, The BR group showed a smear layer-free dentineal surface and an exposed collagen network even before acid etching.

**Conclusions** Brix 3000® was an effective method for removing infected dentine. It can therefore be considered as an alternative to conventional caries removal methods.

KEYWORDS Caries dentine; selective removal caries; enzymatic gel; dentine morphology; SEM

## Introduction

Minimally invasive dentistry includes the identification of risk factors by means of risk assessment tools, the detection of lesions as early as possible and the implementation of preventive strategies and patient education. When the effects of the disease are present in the form of a carious lesion, other therapeutic strategies are required, but in this case less invasive solutions should be chosen, such as remineralisation, the use of therapeutic sealants and restorative care aimed at preserving as much healthy tissue as possible [Tyas et al., 2000]. The terms "minimally invasive dentistry", "ultra-conservative dentistry" and "micro-dentistry", refer to restorative procedures that respect both the dental tissues and patient's comfort. These procedures involve the removal of dentinal caries with the aim of preserving not only healthy tooth tissue but also tissue with remineralisation potential [Featherstone et al., 2012]. Traditionally, the management of deep carious lesions involved removal of the affected dentine until healthy dentine was reached, even if this compromised pulp vitality. Recently, there has been a shift mindset from considering root canal treatment (RCT) as the only alternative in the presence of vital pulp in deep carious lesions. The term vital pulp therapy (VPT) has always been used as a pseudonym for direct pulp capping, but more recent definitions of VPT include strategies aimed at maintaining pulp vitality. [Duncan, 2022]. Nowadays, the issue question is to preserve demineralised dentine (leathery dentine) and even soft dentine in the depth of the cavity [Machiulskiene et al., 2020], with the aim of ensuring a more conservative dental preparation. Residual dentine in the depth of the cavity, prepared using selective removal techniques, is partially demineralised and has intact collagen fibers [Banerjee, 2013]. Conventional methods of caries removal include the use of high-speed diamond or tungsten carbide burs. However, these instruments have several drawbacks, including thermal effects that can damage the pulp, excessive removal of healthy dentine, patient discomfort, and spray production [Banerjee, 2013]. Manual removal with an excavator is an alternative to drilling, although the instrument needs to be sharp and requires the operator to decide how much tissue to remove as it is not self-selective [Neves et al., 2010]. In an effort to overcome these inconveniences, more novel strategies for caries removal have been proposed, such as the use of air abrasion devices, lasers, polymer burs or chemical-mechanical caries removal (CMCR) [Lozano-Chourio et al., 2006]. CMCR has gained interest in research and clinical practice, particularly for the treatment of children or patients with behavioural disorders, because the procedure is simple, painless and overcomes many of the inconveniences of conventional instrument-based caries removal. In addition, when used in conjunction with a metal excavator, CMCR provided the better compromise between caries removal effectiveness and minimally invasive potential [Neves et al., 2010]. CMCR is a minimally invasive technique that also reduces

spray production by using only hand instruments [Banerjee, 2013; Deng et al., 2018] The mechanism of action of CMCR is based on the breakdown of collagen fibres which are partially degraded, facilitating the removal of infected dentine using hand instruments [Schwendicke et al., 2014]. A number of products for CMCR techniques are currently available on the market. Carisolv (RLS Global AB, Sweden), launched in Sweden in 1998, consists of two solutions mixed to form a gel containing sodium hypochlorite, sodium chloride, carboxymethylcellulose and three amino acids (glutamic acid, leucine and lysine). This gel selectively softens the infected dentine, while preserving the demineralised and healthy tissues (Carisolv Technical Sheet). Papacarie (Formula & Ação; Sao Paulo, Brazil) was commercialised in 2003 as a further development of Carisolv and basically contains a proteolytic enzyme (papain) and chloramine. Numerous studies have confirmed the bacteriostatic, bactericidal and anti-inflammatory activity of this combination [Lozano-Chourio et al., 2006; Bottega et al., 2018]. In 2016, Brix 3000® (Brix Medical Science, Santa Fe, Argentina) was launched. It also contains papain, but at a higher concentration than Papacarie (3000 U/mg at a concentration of 10%), which reduces the treatment time. Papain is an endoprotein obtained from the green papaya (Carica papaya). It is similar to human pepsin and has bacteriostatic, bactericidal and anti-inflammatory activity. The papain is bioencapsulated with a neutral pH buffer using encapsulating buffer emulsion (EBE) technology, which enhances its proteolytic action on the infected dentine. The formulation does not contain chloramine; therefore, its activity is more specifically targeted to the denatured proteins of the decayed dentine, leaving the healthy dentine intact[Mahdi et al., 2019]. Polymer burs are made of a polyether-ketone-ketone compound and are designed to selectively remove infected dentine without affecting leathery dentine or the healthy dentine [Schwendicke et al., 2014; Marques et al., 2020]. This is because the hardness of the bur is greater than that of the infected dentine (50 HPN vs 15-20 HPN), on the Knoop scale, but less than that of the healthy dentine (70-90 HPN). These burs are operated at low speed and are manufactured in three sizes [Usha and Ranjani. 2012]. The dentine substrate is a hydrated biological complex with different regional characteristics that can be altered by physiological processes, age, and disease. Significant variations in its architecture can occur depending on the depth and the response to previous aggressions such as carious lesions, cavity preparations, and possible aggressive effects of restorative materials [Sakoolnamarka et al., 2002]. Many studies have evaluated the dentine substrate after removal by conventional methods or CMCR using Carisolv or Papacarie [Lozano-Chourio et al., 2006; Schwendicke et al., 2014]. However, little information is available on the characteristics of the substrate when using other newer methods such as Brix 3000® or mechanical techniques using polymer burs [Divya et al., 2015]. The characteristics of the dentine substrate after carious tissue removal are critical for adhesion. Factors influencing the quality of the bond include the presence of a smear layer created by the excavation process and the hybrid layer resulting from the interaction between the adhesive resin systems and the etched surface [Yildez et al., 2013]. The smear layer produced during cavity preparation is mainly composed of denatured hydroxyapatite and collagen. It must be removed or modified in restorative procedures based on the use of composite resins. The application of orthophosphoric acid at a concentration of 30–40% for 5 seconds effectively removes the smear layer [Scheffel et al., 2016]. When a resin-modified glass ionomer is used as a restorative material, with or without silver diamine fluoride, conditioning the dentine with orthophosphoric acid for 5 seconds does not worsen the adaptation of the material to the dentine walls or the shear bond strength [Ghilotti et al., 2023; Ramachandra et al., 2020]. A number of studies have evaluated the dentine substrate after removal by conventional methods or CMCR using Carisolv or Papacarie [Lozano-Chourio et al., 2006; Schwendicke et al., 2014]. However, little information is available on the characteristics of the substrate when using other newer methods such as Brix 3000® or mechanical techniques using polymer burs [ Divya et al., 2015].

## Material and Methods

The study was approved by the Ethics Committee of the University of Valencia (Spain) with the reference number UV-INV_ETICAA-2479471. The research was conducted according to the standards of the Helsinki Declaration of 1975 revised in 2000. A sample of molars (N=8) was used where extraction was indicated, with ICDAS codes 5 or 6 (5: exposed dentine was observed in a wet tooth, after drying there was visual evidence of open cavitation; 6: the lesion extends to at least the middle third of the dentine). Teeth were removed 3–6 days before processing and stored in buffered saline solution at 4º C.

The tooth surface was cleaned with an ultrasonic tip and pumice, then mounted in silicone bases for handling, and an opening cavity was made in all teeth with a turbine under air and water cooling until the cavity was completely visible. The soft dentine was then removed using each of the study methods according to the procedure described below.

**Tungsten carbide bur (TC)**. The TC bur was used with an angled handpiece (1/1 transmission). A spherical bur (01097K2/009, Komet Dental, Lemgo, Germany) was used for caries removal by working over the cavity walls. Cavity cleanliness was assessed according to Ericson's visual and tactile criteria [Ericson et al., 1999], i.e. using a sharp probe that should not lodge in the dentine and should not produce a pull-back sensation.

**Polymer bur (PB)**. Caries excavation was performed with a Polybur (43119K0/014, Komet Dental, Lemgo, Germany) using an angled handpiece (1/1 transmission) under air and water cooling along the entire cavity wall until the operator confirmed that no further dentinal tissue had been removed.

**Excavator (EX)**. A 17/18 excavator (Dentsply Maillefer, Tulsa, USA) was used. The cavity walls were excavated, removing the soft dentine. Decayed dentine was considered completely removed when no more dentine could be removed from the walls by applying pressure.

**Brix 3000® (BR)**. Brix 3000® (Brix Medical Science, Santa Fe, Argentina) was applied and left to work for 2 minutes, then removed with a non-cutting excavator, using a pendulum motion without pressure. This procedure was repeated 2-3 times until the gel changed colour. The teeth were then removed from the silicone base, the roots were removed and each tooth was axially fractured mesiodistally. Sixteen specimens were obtained, two for each group. Two surfaces per group were used for SEM examination, the other two surfaces were treated with 37% orthophosphoric acid (3M, Saint Paul, USA) for 5 seconds and rinsed. The samples were stored in phosphate buffered saline solution (PBS) until the next stage of the process. Figure 1 summarises the sample preparation, and Table 1 shows the 8 study groups involved. Each sample was rinsed with distilled water and dehydrated in a graded series of 30%, 50%, 70% and 90% ethanol (10 minutes each). Finally, the samples were placed in 100% ethanol for one hour. To neutralise the ethanol, the samples were immersed in acetone for 30 minutes and then in amylacetate other 30 minutes for complete dehydration [Kotb et al., 2016]. To avoid rehydration, all the samples were kept in a dryer until SEM examination. Specimens were coated with a gold-palladium alloy under vacuum pressure

| Group | Caries removal procudere | Group | |
|---|---|---|---|
| TC- A | Tungsten carbide bur | TC-B | TC-A.+ acid etching |
| PB-A | Polymer bur | PB-B | PB-A + acid etching |
| EX-A | Excavator | EX-B | EXA + acid etching |
| BR-A | Brix 3000 | BR-B | BR-A+ acid etching |

TABLE 1 Study groups according to the procedure of caries removal and subsequent dentine treatment.

(Polaron sputter coater SC 7 640. Leica Microsystems, Wetzlar, Germany) and examined on a SCIOS 2 FIB SEM (Thermo Fisher Scientific, Waltham, USA), varying the current and voltage to ensure the best image quality. Images were obtained at different magnifications (x500x, 3,000x, 5,000x, 7,000x, and 25,000x). The surface topography was assessed based on the presence of residual decayed tissue, smear layer, bacteria, and collagen fibers.

## Results

The TC removed eliminated a greater amount of tissue and produced a large smear layer on the cut dentine surface (Figs. 2A and 2B). The subsequent application of orthophosphoric acid largely eliminated these residual traces (both organic and mineralised), but not completely, leaving a smear layer and bacteria on the dentine surface (Figs. 2C and 2D). The PB left a surface covered by a compact smear layer that did not allow visualization of the underlying dentinal surface (Figs. 3A and 3B). The subsequent application of orthophosphoric acid removed the remaining traces in a similar manner to the TC (Figs. 3C and 3D). The EX was unable to completely clean the dentinal surface, leaving a compact smear layer surface (Figs. 4A and 4B). After application of orthophosphoric acid, the smear layer was completely removed and the final result was even better than that obtained with the TC. Under higher magnification, extensively demineralised surfaces were observed with the presence of collagen fibers (Figs. 4C and 4D). Finally, the use of BR produced a demineralised structure with a spongy collagen fiber morphology and a sparse smear layer (Figs. 5A and 5B). This appearance persisted after the application of orthophosphoric acid (Figs. 5C and 5D). In the other groups, collagen fibers could be identified at high magnification (25,000x), although they were more compact than in the Brix 3000® group.

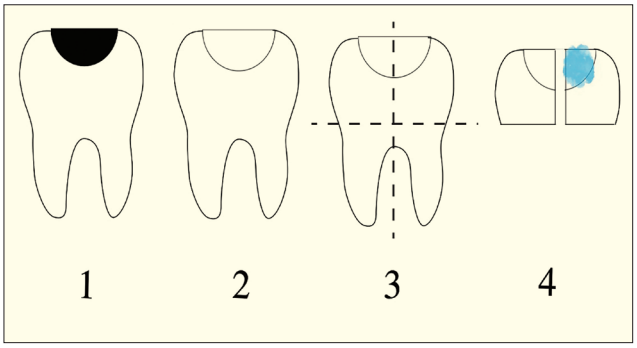


FIG. 1 Representation of the different phases of the procedure: 1- Detection of caries based on the ICDAS II system. 2- Carious lesion removal. 3- Longitudinal and cross-sectioning of the tooth. 4- Application of orthophosphoric acid to one half of the specimen.

## Discussion

Selective caries removal includes conservative techniques to preserve demineralised dentine in the treatment of cavitated carious lesions. In dentinal caries, a distinction is made between an irreversibly damaged soft dentine (characterised by high bacterial

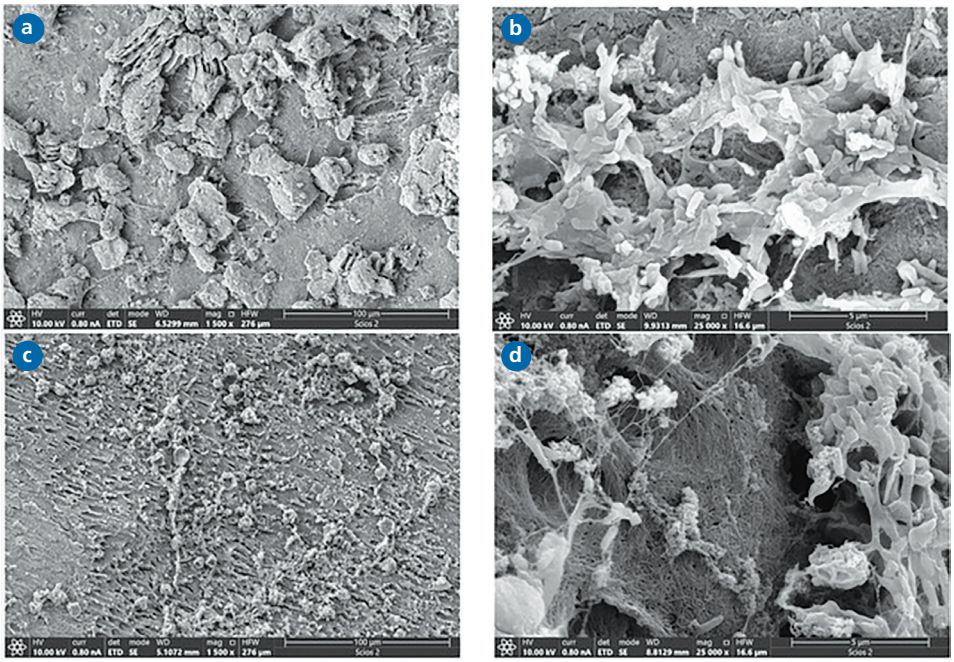


FIG. 2 Dentine removal with tungsten carbide burs (groups TC-A and TC-B).
A) Non-etched surface, x1500. The entire dentine surface is covered by smear layer. Dentinal tubules cannot be seen.
B) Non-etched surface, x25,000. Organic remains and bacteria covering the dentinal substrate.
C) Surface etched for 5 seconds with orthophosphoric acid, x1500. A clean dentine surface can be seen, with some small traces of calcified material, showing tubules that have been longitudinally sectioned.
D) Surface etched for 5 seconds with orthophosphoric acid, x25,000. Demineralized (leathery) dentine with opened tubules, showing both the peritubular and intertubular dentine. Some organic remains and bacteria are observed.

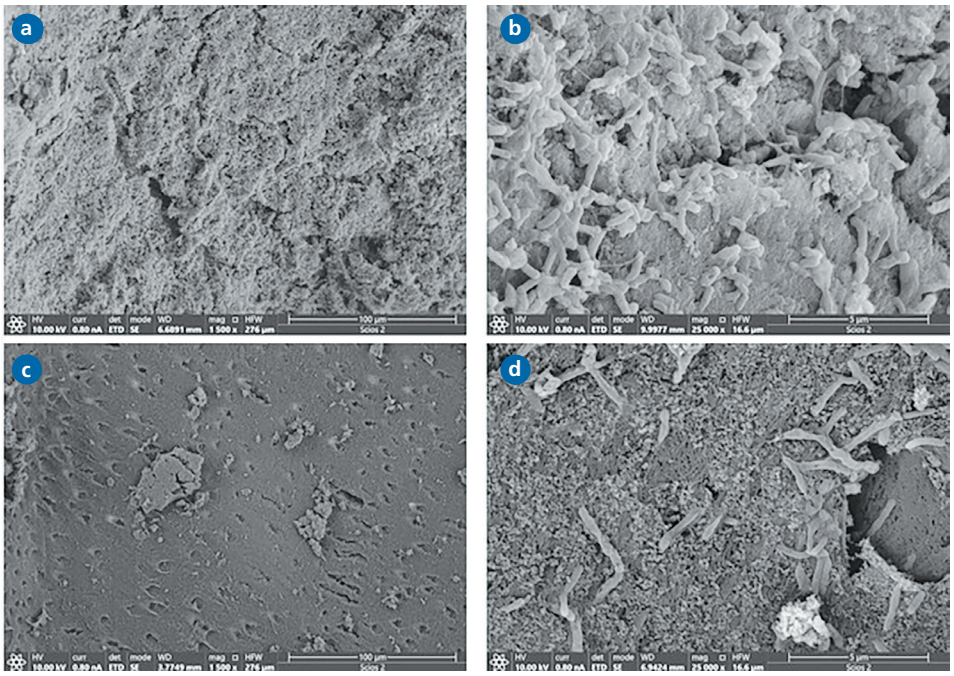


FIG. 3 Dentine removal with polymer burs (groups PB-A and PB-B).
A) Non-etched surface, x1500. A uniform, compact smear layer is observed, covering the entire dentine surface. Dentinal tubules cannot be visualized.
B) Non-etched surface, x25,000. Organic remains and bacteria included in the smear layer.
C) Surface etched for 5 seconds with orthophosphoric acid, x1500. Clean surface with scant smear layer.
D) Surface etched for 5 seconds with orthophosphoric acid, x25,000. Demineralized (leathery) dentine is observed, with a fibrillar network of collagen fibers, mineralized debris and bacteria.

contamination and proteolytic collagen degradation) and a deeper area of demineralised (leathery) dentine that can be repaired under adequate conditions, since the collagen is not denatured [Banerjee, 2013]. The characteristics of the residual dental tissue after caries removal are critical, and the chosen restorative strategy and material must be adapted to avoid complicating the adhesive procedures aimed at restoring the shape and function of the lost tooth structure [Sakoolnamarka et al., 2002]. Factors that influence the quality of the bond between the tooth surface and the dental restoration include the presence of a smear layer produced by the excavation process and the hybrid layer resulting from the interaction between the adhesive resin component and the etched surface [Lozano-Chourio et al., 2006]. The SEM analysis of the substrate left after caries removal led to the rejection of both null hypotheses. This was because the two methods generated distinct surfaces post-caries removal and acid etching. It should be duly noted that not all sample variables could be standardised, owing to the usage of teeth with natural caries. Factors including the age of the patient, the time over which the lesion was formed and presence of tertiary or sclerotic dentine, among others, contribute to the heterogeneity in the morphology of superficial dentine [Banerjee , 2013; Kotb et al., 2016]. The use of rotary instruments, such as PC or PB, creates a uniform smear layer on the surface, which obstructs the entrance to the dentinal tubules. This finding is supported by previous studies [Schwendicke et al., 2014; Yazici et al., 2002]. It has been suggested that the formation of the smear layer by the TC is due to its cutting action, which can cause dentine scaling [Saikaew et al., 2022]. The density of the smear layer,

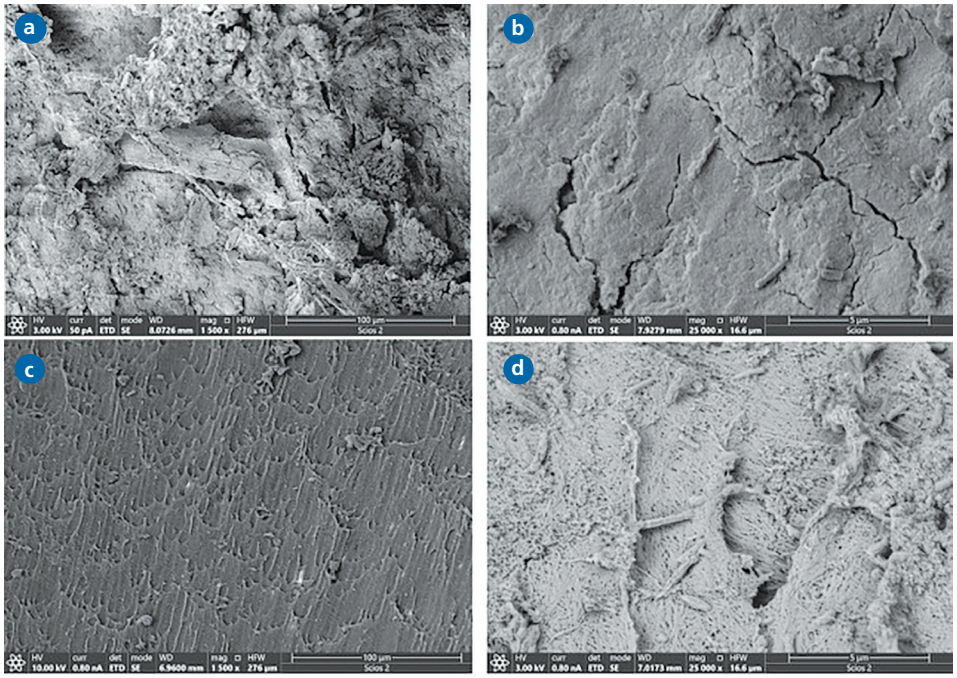


FIG. 4 Dentine removal with the excavator (groups EX-A and EX-B)
A) Non-etched surface, x1500. Compact smear layer is observed covering the dentinal surface completely.
B) Non-etched surface, x25,000. The same as in fig. 4.A.
C) Surface etched for 5 seconds with orthophosphoric acid, x1500. Dentinal surface free of smear layer. Tubular structure can be seen..
D) Surface etched for 5 seconds with orthophosphoric acid, x25,000. A clean dentinal surface is shown with demineralized (leathery) dentine with a fibrillar network.

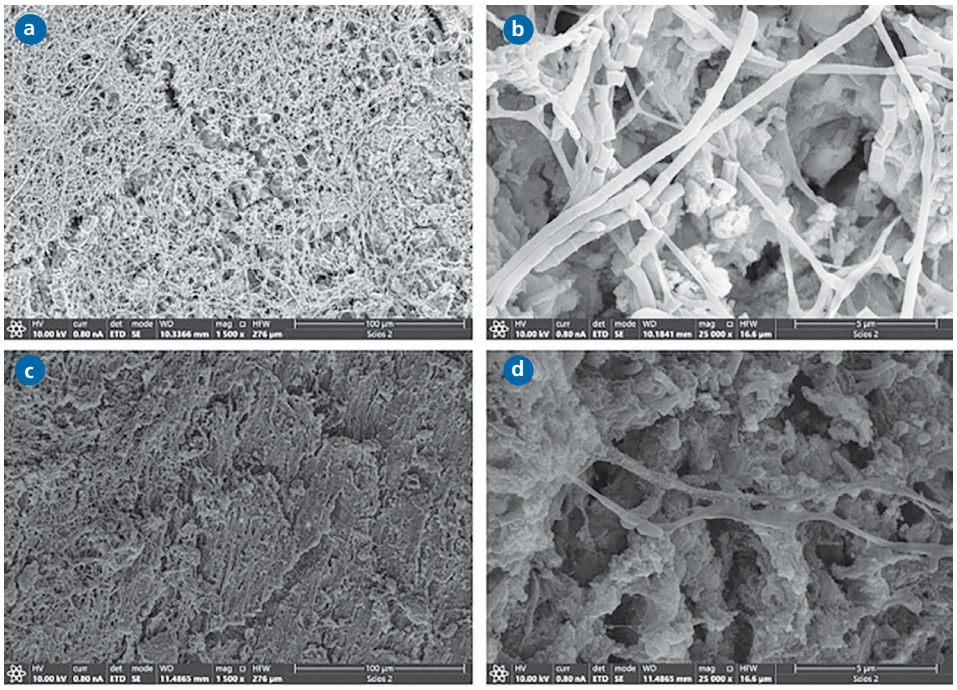


FIG. 5 Dentine removal with Brix 3000® (groups BR-A and BR-B). A) Non-etched surface, x1500. The dentine collagen fiber structure is observed. The underlying layer shows disorganized mineralized dentine in which some dentinal tubules are identified. B) Non-etched surface, x25,000. Collagen fibers can be seen over an irregular calcified dentine. C) Surface etched for 5 seconds with orthophosphoric acid, x1500. Partially demineralized (leathery) dentine with the presence of collagen fibers in some areas. There is no smear layer. D) Surface etched for 5 seconds with orthophosphoric acid, x25,000. Irregular dentinal structure with some fibers.

rather than its thickness, may have adverse effects on effectiveness of adhesive materials, particularly self-etch ones [Sattabanasuk et al, 2007]. Therefore, investigating alternative methods for removing carious dentine tissue is of interest. Several authors have reported that ortophosphoric acid completely eliminates the smear layer when removing caries, regardless of the method used [Oliveira et al., 2003; Saikaew et al., 2022]. In TC-B and PB-B groups, although the smear layer was removed, bacteria were still present, and the remaining surface characteristics were different. In the TC-B group, presence of intertubular and peritubular dentine was observed, suggesting that the TC bur removed both the affected and infected dentine, leaving only healthy dentine [Kotb et al., 2016]. However, it should be noted that certain authors have reported limitations of PB in completely eliminating caries [Lohmann et al., 2019]. However, Usha and Ranjani [2012] compared the efficacy of two polymer burs and found both exhibit total efficiency in eliminating carious tissue in 80% of the samples. Removal of dentine using an excavator leads to the formation of smear a layer, though it is thinner using a TC bur. For excavator usage as a minimally invasive technique, clinicians must be able to distinguish between affected and infected dentine tissue, which can be challenging at times [Duncan, 2022]. Both the traditional excavator and Brix 3000® have proven to be clinically effective in removing caries [Gupta et al., 2022]. The degradation of collagen in infected dentine is caused by CMCR techniques. [Beeley et al., 2000]. Enzymatic properties of Brix 3000® contribute to its efficacy. Coinciding with our own observations, other authors have reported that the use of this product leads to the emergence of exposed dentine tubules, absence of dentine smear formation, with melted-like peritubular dentine, and the presence of microorganisms [Donmez et al., 2022]. Not all CMCR techniques result in the absence of smear layer. Chittem et al. reported the formation of smear layer obliterating the tubules when Papacarie® was used [Chittem et al., 2015]. Additionally, some authors have reported greater bacterial presence following the use of Papacarie® compared to the use of a TC bur [AlHumaid et al., 2020]. These variations could be ascribed to the quantity of papain (3000 U/mg at a 10% concentration), as well as the EBE technology used in Brix 3000®, which enhances the proteolysis of collagen fibers in decomposed tissue [Alkhouli et al., 2020]. The low presence of bacteria noticed in our research after the use of Brix 3000® aligns with a microbiological study wherein significant statistical variations in the quantity of colony-forming units (CFUs) were documented when using other procedures to remove caries [Divya et al., 2013; Inamdar et al., 2020]. Dentine topography plays a critical role in the adhesive process of dental restoration [Sakoolnamarka et al., 2002]. The observation of well-preserved collagen fibers after applying Brix3000® implies that a more dependable self-etch adhesive may be feasible. This is due to ortophosphoric acid, which is used to remove the smear layer, potentially leading increased tooth sensitivity [Lozano-Chourio et al., 2006], whereas applying Brix 3000® results in practically no smear layer on the dentine surface. Chemical-mechanical caries removal procedures are highly applicable in paediatric dentistry and for special needs patients, because it reduces pain and anxiety caused by rotary instruments, effectively disinfect and remove denatured dentine [Inamdar , et al., 2020; Gupta et al., 2022], leaving a smear-free substrate with exposed collagen. Not everyone is prepared for complex dental treatment at an early stage, particularly children or people with physical or behavioural limitations. The CMCR procedures generate less stress for the child and the family and prepare the child and the family for more complex treatments in the future. This approach generates confidence in the parents, who appreciate that our aim is not only the child's teeth or mouth, but their health and well-being [Beretta, et al., 2022; Beretta, 2022; Beretta, et al., 2023]. The present study has limitations, firstly the standardization of the samples. Two halves of the same tooth were used, one after caries removal and the other after acid etching. However, it was not possible to determine the viability of bacteria not removed by mechanical procedures using SEM. However, this area of research aligns with the principles of minimally invasive dentistry, necessitating future investigation into aspects not assessed in this study, such as the survival of remaining microorganisms and the adhesive interface with different systems and materials.

## Conclusion

In conclusion, according to the results of the study, the

mechanical methods left the dentine surface entirely covered with smear layer with occluded tubules. In contrast, the chemo-mechanical removal method using Brix 3000® resulted in only a small amount of smear layer, with exposed tubules and sponged collagen fibers. After acid etching, all groups showed some remaining smear layer, with visible collagen fibers and exposed tubules. Nevertheless, there were still areas covered with smear layer and bacteria in the mechanically treated groups. Based on the results of the present study, the use of chemical-mechanical methods for selective caries removal could serve as a viable alternative in treating deep caries lesions. Additional research should be conducted to determine the most appropriate adhesive techniques for restoration.

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