Dentine microhardness after different methods for detection and removal of carious dentine tissue

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ABSTRACT

There are several methods for identifying carious dentinal tissue aiming to avoid removal of healthy dentinal tissue. Objectives: The purpose of this study was to test different methods for the detection of carious dentinal tissue regarding the amount of carious tissue removed and the remaining dentin microhardness after caries removal. Material and methods: The dentin surfaces of 20 bovine teeth were exposed and half of the surface was protected with nail polish. Cariogenic challenge was performed by immersion in a demineralizing solution for 14 days. After transverse cross-section of the crown, the specimens were divided into four groups (n=10), according to the method used to identify and remove the carious tissue: "Papacárie", Caries-detector dye, DIAGNOdent and Tactile method. After caries removal, the cross-sectional surface was included in acrylic resin and polished. In a microhardness tester, the removed dentin thickness and the Vickers microhardness of the following regions were evaluated: remaining dentin after caries removal and superficial and deep healthy dentin. Results: ANOVA and Tukey's test (α=0.05) were performed, except for DIAGNOdent, which did not detect the presence of caries. Results for removed dentin thickness were: "Papacárie" (424.7±105.0; a), Caries-detector dye (370.5±78.3; ab), Tactile method (322.8±51.5; bc). Results for the remaining dentin microhardness were: "Papacárie" (42.2±10.5; bc), Caries-detector dye (44.6±11.8; abc), Tactile method (24.3±9.0; d). Conclusions: DIAGNOdent did not detect the presence of carious tissue; Tactile method and "Papacárie" resulted in the least and the most dentinal thickness removal, respectively; Tactile method differed significantly from "Papacárie" and Caries-detector dye in terms of the remaining dentin microhardness, and Tactile method was the one which presented the lowest microhardness values.

Key words: Dentin. Dental caries. Lasers. Hardness.

INTRODUCTION

A carious dentinal lesion has been described as one consisting of two distinct layers with different ultrastructural and chemical characteristics. The outer layer is contaminated with bacteria. As the organic matrix is substantially degraded and cannot be remineralized, this layer of caries-infected dentin must be removed. The inner layer is partially demineralized but not contaminated with bacteria. As there is only limited collagen degradation, the inner layer of caries-affected dentin can be remineralized and should be preserved[6].

There are many methods for identifying carious dentinal tissue aiming to avoid removal of healthy dentinal tissue. Among them, it can be cited the tactile method using a dental explorer, the caries-

detector dyes and, more recently, substances such as gels based on papain and laser fluorescence devices such as DIAGNOdent (Kavo, Biberach, Germany).

The tactile method, using a dental explorer, is still the most widely used in dentistry because it does not require sophisticated equipment or even the use of any other product. So, it is a reliable method, with low cost and easy application.

Caries-detector dyes were launched in 1972 and intended to enhance complete removal of infected carious dentin without over-reduction of healthy dentin. Subsequent clinical trials judged that there was no correlation of the dye-stained material with infection but rather with lower levels of mineralization, with or without infection[17].

"Papacárie" (Fórmula e Ação, São Paulo, SP, Brazil) was launched in 2003 as a new method for chemomechanical removal of caries, focused on tooth preservation by a minimally invasive technique. It is composed by papain, chloramine and blue toluidine. Papain is supposed to interact with the collagen exposed by dissolution of dentin minerals by bacterial action, softening the infected dentin and enabling its removal by blunt hand instruments, with no need for anesthesia and use of rotary instruments[29].

The laser fluorescence device DIAGNOdent has been developed for objective caries diagnosis2[2,31,32]. This device works based on the principle that the laser fluorescence emitted from carious surfaces is greater than that emitted from sound surfaces[3]. The fluorescence emitted from a test surface is displayed as numerical values ranging from 0 to 99, with deeper carious lesions producing higher values[12,22,24,31]. The numeric value that indicates the right time to stop caries removal still needs further studies. However, some studies have shown that values between 11 and 20 seem to be the most appropriate[13,15,21,35]. More studies are necessary to assess whether the methods mentioned above are able to identify only the outer layer of carious dentin, contaminated with bacteria.

Thus, the purpose of this study was to test four methods for detecting carious dentin tissue created artificially in bovine teeth through a demineralizing solution with no bacterial infection, and to evaluate the remaining dentin microhardness resulting from the use of these methods.

MATERIAL AND METHODS

The research project was approved by the Ethics Committee, São José dos Campos School of Dentistry, UNESP (protocol number 005/2010). Twenty bovine incisors had their roots amputated in a high speed lathe (Nevoni, São Paulo, SP, Brazil) for the use of their crowns. The pulp chamber access was performed with high speed round diamond burs for removal of pulp debris using endodontic files and to allow further standardization of buccal dentin thickness. The selected teeth were stored in distilled water at 4°C, exchanged periodically until the time of use, not exceeding a period of 6 months[14].

The buccal enamel was worn in a rotary polisher (Extec Corp., Enfield, CT, USA) with 80-grit abrasive paper (Struers, Ballerup, Denmark), at 300 rpm, until exposure of a dentin area of 1 cm^2. The remaining dentin thickness was standardized at 1.5 mm by the use of a thickness meter. Final polishing of the exposed dentin surface was done with 160-grit abrasive paper.

The opening on the lingual surface of the crown and the region of root amputation were both protected with red utility wax. The buccal dentin was positioned at the bottom of a silicon mold (Silibor, Classic, São Paulo, SP, Brazil) to be embedded in chemically activated acrylic resin (Jet, Clássico, São Paulo, SP, Brazil), resulting in an acrylic resin cylinder with dentin surface exposed.

Next, dentin surface was subjected to a cariogenic challenge by immersion in 5.75 mL of a demineralizing solution prepared with 50 mM of acetate buffer pH 5.0, 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4 and 0.5 ppm of fluoride as NaF[9,34] for 14 days, at room temperature. The solution was changed every 7 days. Prior to the immersion of the specimens, half of dentin surface was covered with two layers of nail polish (Colorama, São Paulo, SP, Brazil) to allow the maintenance of a sound dentin surface after dentin cariogenic challenge (Figure 1). The period of immersion was defined by a pilot study in which the carious dentinal tissue thickness was evaluated daily by a microhardness tester microscope (Microhardness Tester FM-700, Future-Tech Corp., Tokyo, Japan).

Next, transverse cross-section of the crowns was performed in a high speed lathe (Nevoni,
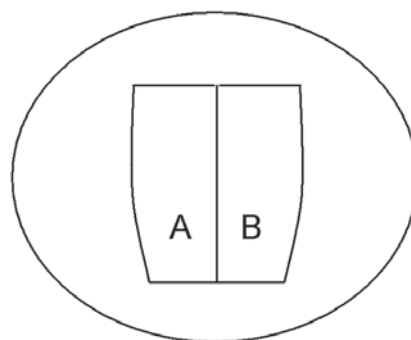
Figure 1- Scheme for the vestibular dentin exposed and embedded in acrylic resin. A- surface submitted to cariogenic challenge (sound dentin); B- surface covered with nail varnish (healthy dentin)

Group	Manufacturer	Composition	Application Technique
G1 "Papacárie" + Dentin curette	"Fórmula e Ação", São Paulo, SP, Brazil SSWhite, Duflex, Rio de Janeiro, RJ, Brazil	Papain, chloramine, toluidine blue, salts, conservative, thickener, vehicle qs	Leave product act for 30 s, scrap softened dentin with blunt curette. Reapply until obtaining hard dentin
G2 + Low-speed #6 round steel bur	"Videocárie", Iodontec, Porto Alegre, Rio Grande do Sul, Brasil JET, Beavers Dental, Morrisburg, ON, Canada	Basic fucsin, solvent, vehicle qs	Dry well, apply dye with a cotton ball, wait 10 s, rinse, remove dentin stained in red. Repeat until there is no more stained dentin
G3 DIAGNOdent + Low-speed #6 round steel bur	Kavo, Biberach, Riss, Germany JET, Beavers Dental, Morrisburg, ON, Canada	_____	Calibrate the device and approximate the tip to the carious surface. The alert sound indicates that there is still carious tissue
G4 Dental explorer + Low-speed #6 round steel bur	SSWhite, Duflex, Rio de Janeiro, RJ, Brazil JET, Beavers Dental, Morrisburg, ON, Canada	_____	Stop removal when dental explorer checks hard dentin

Figure 2- Distribution of groups, composition and manufacturer's recommendations of use
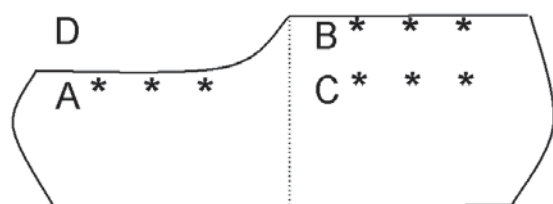
Figure 3- Points and regions of microhardness assessment in dentin. A- remaining sound dentin; B- superficial healthy dentin; C- deep healthy dentin; D- thickness of dentin removed

São Paulo, SP, Brazil). The resulting 40 specimens were randomly divided into four groups (n=10), according to the method used for identification and removal of caries (Figure 2).

After caries removal by one of the methods mentioned, the surface on the cross-section was included in chemically activated acrylic resin, placing the cut surface facing the bottom of a cylindrical silicon mold. Cross-section surface was taken to a rotary polisher with 600-, 1200-, 2400- and 4000- grit abrasive papers (Struers, Ballerup, Denmark) until achieving a smooth and brightening surface, required for microhardness evaluation (Microhardness Tester FM-700, Future-Tech Corp., Tokyo, Japan).

The microhardness test was performed on each of the following regions: remaining dentin after caries removal and superficial and deep healthy dentin (Figure 3). The distance between the three indentations of each region was 10 µm. Nine microhardness values were obtained for each specimen. Indentations on deep healthy dentin followed the same horizontal alignment as the remaining dentin. The mean microhardness value was calculated for each region analyzed for subsequent statistical analysis.

Microhardness analysis also allowed obtaining the thickness of dentinal carious tissue removed in each specimen, and these data were also analyzed statistically.

The perfect parallelism of the analyzed area to the microhardness tester base was managed by the use of a metal appliance specific for this, which was adaptable to the microhardness tester. A Vickers indenter was used with a static load of 25 kgf and dwell time of 10 s, defined by a pilot study.

Data from removed dentin thickness (in µm) and microhardness (VH) had a normal distribution, as verified in scatter plots, in which the dispersion of observed data tended to a straight up. Parametric one-way ANOVA and Tukey's test were performed for comparison of means between groups. The program used for the statistical analysis was the BioStat 5.0. The significance level was set at 5%.

RESULTS

DIAGNOdent was the only method that did not identify the presence of carious dentin. The maximum value provided by this device in all specimens analyzed was 5, which does not indicate

removal of any tissue. Thus, this group did not participate in the statistical analysis.

In relation to the removed dentin thickness in each group, there was statistically significant difference between "Papacárie" and Tactile groups (p=0.03) (Table 1).

In relation to remaining dentin microhardness, the Tactile group presented significantly lower values than "Papacárie" and Caries-detector dye groups (p=0.0001) (Table 2).

There were no significant differences in microhardness between the dentin remaining after caries removal and superficial healthy dentin in any of the groups. Tactile group was the only one that presented statistically significant difference between the remaining dentin and deep healthy dentin. In the other groups, the remaining dentin microhardness was statistically similar to deep healthy dentin microhardness. It is important to remind that indentations on deep healthy dentin were in the same horizontal alignment as the indentations on remaining dentin.

Table 1- Mean, standard deviation and Tukey's test (5%) for dentine thickness removal data in each group (in µm)

Method	Mean	Standard deviation	Homogeneous groups
"Papacárie"	424.7	105.0	a
Caries-detector dye	370.5	78.3	ab
Tactile	322.8	51.5	bc

The groups followed by the same letters do not show statistically significant difference

DISCUSSION

Current management of caries involves non-invasive techniques and maximum preservation of tooth structure. Differentiation between heavily infected outer carious dentin and demineralized affected inner dentin reduces the risk of pulp exposure, maximizing the reparative potential[23]. Different layers of dentin carious lesions have been classified by clinical and laboratory techniques[1,8], but recommendations may conflict or overlap.

In this study, artificial caries was created in dentin, using a demineralizing solution with no bacterial infection to test if the caries detection methods would detect the presence of caries, which, in fact, would be predominantly a demineralized tissue, not contaminated with bacteria. As it is known, these methods should recommend the removal only of the outer layer of carious dentin tissue, not contaminated with bacteria, so it would be expected no identification of the presence of carious dentin tissue.

However, only DIAGNOdent did not detect the presence of caries, as the maximum value provided by the device was 5, which does not indicate the presence of caries. Some recent studies reported that one must consider the presence of caries when the values obtained by the device are up to the interval between 11 to 20[13,15,21,35]. Thus, it was not possible to take samples of the removed dentin thickness and the remaining dentin microhardness in this group.

DIAGNOdent is a caries diagnostic device that uses a laser and the fluorescent properties of tooth substance, and indicates the condition of carious dentin in pit-and-fissure areas and on smooth surfaces with numerical values[10]. When the reflection fluorescent spectrum in the near-infrared region in tooth substance irradiated

Table 2- Mean, standard deviation and Tukey's test (5%) for dentine microhardness (in VH)

Method	Dentin region	Mean	Standard deviation	Homogeneous groups
"Papacárie"	Remaining	42.2	10.5	bc
	Superficial healthy	47.5	13.7	abc
	Deep healthy	59.3	7.2	ab
Caries-detector dye	Remaining	44.6	11.8	abc
	Superficial healthy	56.5	14.2	abc
	Deep healthy	60.8	12.5	a
Tactile	Remaining	24.3	9.0	d
	Superficial healthy	39.6	13.3	cd
	Deep healthy	45.0	17.6	abc

The groups followed by the same letters do not show statistically significant difference

by a semiconductor red laser was investigated, differences in the reflection fluorescent strength were detected between sound tooth substance and carious tooth substance[18]. This principle has been applied to DIAGNOdent, which indicates the relative values of fluorescent light emitted from tooth substance areas irradiated by a semiconductor red laser with 655 nm wavelength and an output lower than 1 mW via the tip[10]. Hosoya, et al.[12] (2001) have used DIAGNOdent values to guide carious dentin removal with rotary cutting instruments and have found that the values gradually decrease as carious dentin is removed.

The fact that DIAGNOdent did not provide numerical values indicative of the presence of carious dentin tissue in this study makes sense as it is known that these values are influenced to the fluorescence emitted from bacterial metabolites[11], and are also related to the amount of organic matrix[24,31]. Moreover, Iwami, et al.[15] (2004) observed that no bacteria were detected at DIAGNOdent value less than 15.6±1.2, and the values obtained in this study did not exceed 5.

In the other groups – "Papacárie", Caries-detector dye and Tactile – there was identification of the presence of caries, and the removed dentin thickness differed significantly between "Papacárie" and Tactile groups. The Tactile method was the one that least removed dentin in thickness and "Papacárie" was the one that most removed dentin in thickness, matching statistically the Caries-detector dye group.

It is known that there is still much discussion in the literature regarding the actual efficiency of caries-detector dye and "Papacárie" to identify only the outer layer of carious dentin, which should be removed. Caries-staining products have been developed to assist clinicians during caries removal. Although the biomechanical principle of staining carious dentin has been reported[19,20,27], it remains unclear what characteristics of the lesion are stained, or how staining is related to microstructural features of various caries lesion zones. Not all stainable dentin is infected[16], but the absence of stain does not insure bacterial elimination[1].

"Papacárie" is a papain-based gel product for selective removal of dentin caries. However, in this study, "Papacárie" has detected the presence of carious tissue created artificially without bacterial contamination. According to the literature, papain should act only on the infected dentin that lacks alpha-1-antitrypsin, a substance that inhibits its proteolytic action on healthy tissue[29]. Additionally, there are some advantages of "Papacárie" that should be regarded such as the reduction of discomfort and pain, its antibacterial properties and its potential for not producing smear layer on the surface of a prepared cavity[25].

In this study, "Papacárie" was the one that yielded the highest mean in thickness of caries removal, matching the thickness removed with the aid of caries-detector dye. As no bacterial contamination was used to generate artificial carious dentin tissue, it was expected that the indication for tissue removal by the use of these products would be very insignificant or would not even exist.

Previous studies have shown that carious dentin tissue microhardness increases from the outer to the inner layer[3,7,26,30], so it was decided to study this parameter on the different regions of dentin in this study. Three indentations were made on each region of dentin as it is not a homogeneous tissue[4]. Microhardness analysis has been used as a method to assess loss and reincorporation of minerals to the dental tissue, because the reduction in the numerical hardness value presents a linear relation to mineral loss[5].

Regarding remaining dentin microhardness, the Tactile group differed significantly from the other two groups and was the one with the lowest microhardness values among the three groups. The lower remaining dentin microhardness mean value of the Tactile group is supported by the fact that group presented the lowest removed dentin thickness mean. Thus, there should have remained greater thickness of carious dentin with potential to be remineralized, what explains the lower microhardness values. Pugach, et al.[28] (2009) showed that nanohardness values for intertubular dentin increased from the pink zone to the apparently normal dentin layer (outer to inner). Angker et al.[2] (2004) also found out that mechanical properties across dentin carious lesions decreased as the lesion surface was approached.

Since none of the groups showed significant difference between the remaining dentin microhardness and superficial healthy dentin, it is quite obvious that caries removal by the studied methods might have been excessive, since it is known that the non-contaminated inner dentin layer can be remineralized over time without needing to be removed[6].

Tactile group was the only one that showed statistically significant difference between deep healthy dentin and remaining dentin. In the other groups, remaining dentin microhardness values statistically matched deep healthy dentin ones. It should be bear in mind that indentations on deep healthy dentin were in the same horizontal alignment as those on the remaining dentin.

Studies using dentin caries, artificial or natural, but with bacterial infection, still need to be performed to elucidate the efficiency of methods for identification and removal of dentin caries. Thus, it would be possible to obtain more consistent

information about the remaining dentin after caries removal with these methods, as well as to know more about the characteristics of the removed dentin.

CONCLUSIONS

Among the methods used and according to the methodology of this study, DIAGNOdent was the only one that did not detect the presence of carious dentin; The tactile method was the one which least removed dentin in thickness and "Papacárie" was the one which most removed dentin in thickness, matching statistically the Caries-detector dye group; Regarding the remaining dentin microhardness after caries removal, there was a statistically significant difference only between Tactile and Caries-detector dye groups, and the Tactile group showed the lowest microhardness values among the three groups; Clinicians should be alert to the use of methods for detection of carious dentinal tissue because some of them may indicate the need for removal of healthy dentin.

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