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ORIGINAL RESEARCH

Is It Possible to induce Artificial Caries-affected Dentin using the Same Protocol to Primary and Permanent Teeth?

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ABSTRACT

Aim: This *in vitro* study compared the mineral loss of natural and artificially-created caries-affected dentin in primary and permanent teeth using the same protocol to induce caries lesions.

Materials and methods: Twenty molars presenting natural occlusal dentin caries lesions (10 primary–PriC and 10 permanent–PermC; control group), and 20 sound molars (10 primary – PripH and 10 permanent–PermpH; experimental group), were selected. Occlusal cavities were prepared in teeth of the experimental group that were submitted to pH-cycling for 14 days to simulate caries-affected dentin. All specimens were longitudinally sectioned and prepared in order to obtain Knoop microhardness values from 15 to 250 µm depth, starting in bottom of center of natural lesions or cavities. The microhardness (KHN) data were submitted to three-way repeated measures analysis of variance (ANOVA) and Tukey's tests (α = 0.05).

Results: Considering all depths, there was no statistically significant differences ($p > 0.05$) between the mineral loss of the control (PriC = 30.9 $\pm$ 6.4 and PermC = 40.8 $\pm$ 8.6) and experimental (PripH = 27.3 $\pm$ 11.1 and PermpH = 35.5 $\pm$ 14.0) groups, neither between primary and permanent teeth.

Conclusion: The mineral loss of the artificially-created caries-affected dentin is similar to that from naturally developed dentin caries lesions.

Clinical significance: The pH-cycling model may be a suitable method to simulate caries-affected dentin in both permanent and primary teeth.

Keywords: Dental caries, Deciduous dentition, Dentin, Hardness, Laboratory research, Permanent, Tooth.

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INTRODUCTION

The actual concepts of minimal intervention (MI) dentistry are characterized by a better understanding of the caries process and ultraconservative approaches for treating cavitated dentin lesions.[1] In clinical situation, the bonding surface that is most frequently encountered after caries excavation consists of caries-affected dentin (CAD), which has prompted the investigation of restorative materials' performance on this substrate.

Contemporary studies have evaluated not only the bond strength of different adhesive materials to CAD, but also the resin-dentin bonds stability on this substrate and different caries excavation techniques.[2-8] Nevertheless, the large variability in activity status, shape, size, depth of natural CAD as well as structural differences within different carious zones create technical difficulties in obtaining this substrate in a standardized way for testing materials and new techniques.[2,9] This is especially critical for primary teeth, due to their reduced thickness of enamel and dentin, that often leads to pulp chamber involvement. Furthermore, several structural characteri stics of natural carious dentin can be related to controversial results regarding the adhesion to CAD. Some investigations

have showed lower bond strength values to CAD as compared to sound dentin, while others showed higher bond strength to CAD or similar values between substrates.[10-14]

In an attempt to overcome difficulties when using natural caries-affected dentin, artificial methods have been proposed to create *in vitro* caries-like lesions.[4,15-18] The pH-cycling dynamic model has been widely employed for the development of enamel lesions, the reason for which it was proposed, and nowadays, to simulate artificial caries dentin.[15,19,20]

Marquezan et al verified that pH-cycling promotes a caries-affected dentin layer with superficial demineralization, with similar hardness values as naturally CAD in primary teeth.[15] It has been evidenced that primary and permanent dentin present chemical and microstructural differences.[21] In view that the concentration of calcium and phosphate in the peritubular and intertubular dentin of permanent teeth is higher as compared to primary ones, studies are necessary to evaluate if the same caries induction protocol can be applied on both substrates.[21]

Since microhardness test has been used to indirectly evaluate mineral content of dental substrates, the aim of this *in vitro* study was to compare the mineral loss of natural and artificially-created CAD in primary and permanent teeth using the same protocol to induce caries lesions, by hardness evaluation.[2,22]

MATERIALS AND METHODS

Selection and Teeth Preparation

This research protocol received approval from the research ethics committee. Twenty carious and 20 sound human teeth, half of them primary (Pri) molars and the other half permanent (Perm) molars, were selected from a tooth bulk. Teeth were disinfected with 0.5% chloramine and stored in distilled water at 4ºC until use.

Primary and permanent molars presenting caries lesions on the occlusal surface with 2 ± 0.5 mm depth from amelodentinal junction measured with a periodontal probe, after longitudinal cut, were included in this study and considered as naturally caries-affected dentin group (control group: C group).

Teeth from C groups were longitudinally sectioned in the center of the lesion with a water-cooled low-speed diamond saw (Extec 12205, Extec Co, Enfield, USA), mounted in a cutting machine (Labcut 1010, Extec Co, Enfield, USA), and then immediately prepared for the hardness evaluation.

Sound primary and permanent molars that comprised the experimental groups (pH groups) were submitted to a pH-cycling model to simulate artificial caries-affected dentin. Occlusal cavities (2 × 4 × 2 mm) were prepared using a diamond bur (2094 KG Sorensen, São Paulo, Brazil) with a high-speed handpiece with water spray. Teeth had their cervical portions sealed with quick-setting epoxy resin (Araldite Hobby, Ciba Especialidades Químicas Ltd., São Paulo, SP, Brazil), and rendered waterproof with two layers of acid-resistant nail polish (Colorama Maybelline Ltd., São Paulo, SP, Brazil), with the exception of inner part of the cavities.

Specimens were then individually immersed in 10 ml of demineralizing solution (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 50 mM acetic acid; adjusted pH of 4.8) for 8 hours and in the same volume of remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 mM KCl; adjusted pH of 7.0) for 16 hours.[15] After immersions, the teeth were rinsed with deionized water. This procedure was carried out for 14 days at room temperature without agitation. At the end of pH-cycling period, teeth were longitudinally sectioned in the center of the cavities in the same way as for teeth of C groups.

Hardness Evaluation

Two halves of each teeth were embedded in epoxy resin (Buehler Ltd., Lake Bluff, IL, USA), so that the area to be analyzed remained exposed. Polishing was performed using a polishing cloth (Ecomet 4, Buehler, Lake Bluff, IL, USA) with 320, 480, 600, 1200, 2500 and 4000-grit silicon carbide abrasive papers, and final polishing was made with diamond paste of 1 µm and 0.25 µm (Buehler Ltd., Lake Bluff, IL, USA). After the last stage of polishing, the specimens were ultrasonically cleaned to remove eventual residues.

Fifteen indentations were made in the center of the caries lesions (C groups) or cavities (pH groups) and 100 µm on each side, at depths of 15, 40, 100, 150 and 250 µm using a Knoop indenter, with a static load of 25 gm for 30 seconds coupled with HMV II microhardness tester (HMV II, Shimadzu, Kyoto, Japan).

STATISTICAL ANALYSIS

The experimental unit in this study was the tooth. Thus, microhardness values means for all experimental groups was expressed as the average of the 10 teeth used per group. Additionally, from each specimen, at each depth from the pulp floor, the average was obtained considering the three measurements (center and 100 µm on each side).

The normal distribution of the data was confirmed using Kolmogorov-Smirnov test. Microhardness data were subjected to three-way repeated measures analysis of variance (ANOVA), using a factorial design with group (natural CAD *vs* artificial CAD-pH-cycling), indentation depth and tooth type (primary and permanent teeth) as

variables. The clustered variable was the indentation depth. Tukey's HSD multiple comparisons statistical test at a 0.05 significance level was used. Statistical analysis was performed with GMC software, version 7.7.

RESULTS

Graph 1 summarizes the microhardness (KHN) values as function of depth for all experimental groups.

The results of the microhardness testing did not show statistically significant differences ($p > 0.05$) in mineral loss between natural and artificially-created CAD. The same way, the mineral loss was similar ($p > 0.05$) between primary and permanent teeth in all depths.

DISCUSSION

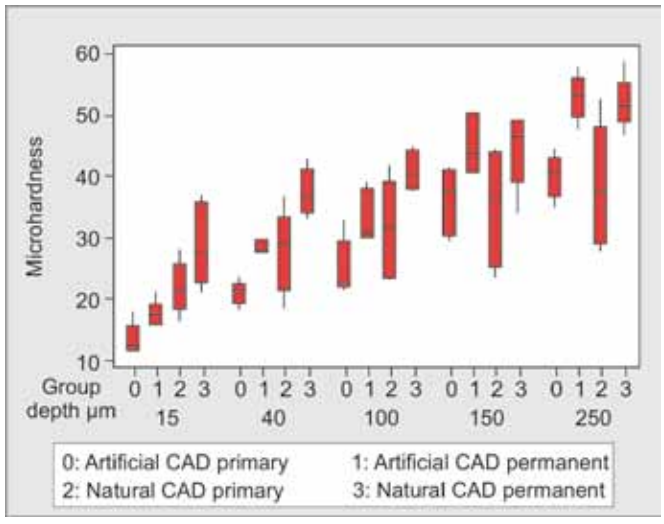
The irregular characteristics of natural caries dentin associated to difficulty in obtaining this substrate in a standardized way for *in vitro* investigations has instigated the development of different models to induce caries-like lesions.

Considering the influence of chemical differences between primary and permanent dentin in development pattern of artificial caries lesions, this is a pioneering investigation that assessed if same protocol using pH-cycling model could be applied to both substrates.

In our study, the mineral loss was similar between primary and permanent teeth, irrespective of the indentation depth. Despite of the discrepancies in mineral content, this result validates the use of the same protocol (pH-cycling for 14 days, using a demineralizing solution with adjusted pH of 4.8) to artificially induce CAD in both substrates.

The mean microhardness values were similar between natural and artificial caries-affected dentin groups. However, the standard deviations were greater in C groups, especially in primary teeth (Graph 1). These findings reflect the variability of the naturally developed dentin caries lesions which occur over long periods of time, in contrast to the artificially-created CAD, where the lesion was created in a short period of time under controlled conditions. These findings corroborate a previous study that compared the mineral content of natural and artificially-created caries-affected permanent dentin, by an another pH-cycling model, at different depth levels until 150 µm.[17] Recently, Joves et al also demonstrated that natural and artificial caries-affected dentin tissues of permanent teeth were superficially comparable in intertubular nanohardness.[23]

Among the chemical and microbiological methods of dentin caries induction, the pH-cycling model has been considered the most appropriate design to simulate



Graph 1: Mineral loss as function of depth for all experimental groups ($p > 0.05$)

the carious process, since it promotes alternate periods of demineralizing and remineralizing; although the real duration these periods in the oral environment is unknown.[24,25] According to a previous study, microbiological method is more aggressive than pH-cycling in primary teeth, especially until 100 µm depth.[15] Additionally, this method seems more indicated to simulate an infected layer of dentin caries lesion, not caries-affected dentin, which is considered the appropriate substrate to perform bonding approaches in clinical situations.

In turn, the lowest hardness values obtained for artificially-induced CAD were also similar to that reported by Fusayama and Terashima for transparent dentin, which exists beneath carious lesions.[26,27] It is important to highlight that despite the absence of statistically significant difference among the depths evaluated, at 250 µm depth the values were near to sound dentin hardness. Based on that, this method of caries induction seems to promote a slightly superficial demineralization. Marquezan et al also demonstrated that pH-cycling method provided similar hardness values in comparison to naturally caries-affected dentin of primary teeth until the depth 40 µm, and lower values than sound dentin until depth of 200 µm.[15]

Despite similarity in mineral loss between types of caries-affected dentin, the formation mechanisms are different. Natural caries lesions show two layers of the carious dentin: a portion of soft and moist dentin highly infected for bacteria, and a layer of affected dentin.[28] The caries-affected dentin layer, although is demineralized and contains dentinal tubules occluded by acid-resistant mineral crystals, shows viable odontoblastic processes and normal collagen, suitable of remineralization.[26]

One limitation of the simulation of artificial CAD in extracted or exfoliated teeth is that the reaction of the dentin pulp complex to the demineralization process, represented by tubular occlusion with acid-resistant

whitlockite minerals, is not present, differing somewhat with the clinical situation. Thus, we speculate that the artificial caries-affected dentin presents such aspects to an acute natural caries lesion.

Besides substrate demineralization, *in vivo* lesion formation in dentin involves the degradation of the exposed organic matrix by metaloproteinases (MMPs) activated by acidic conditions and proteinases from microorganisms of the carious process.[29,30] It must be emphasized that *in vitro* caries-like lesions cannot accomplish all factors involved in the carious process. When pH-cycling method is considered, the demineralization of the substrate is characterized by the absence of bacteria. Nevertheless, the greatest advantage of this method is the possibility of the fast and reliable formation of the carious lesions, providing more homogenous dentin surfaces.[24,25] Therefore, the pH-cycling method seems be a suitable method to simulate CAD for several purposes, mainly for bond strength analysis, irrespective of tooth type (primary or permanent).

CONCLUSION

The mineral loss of the artificially-created caries-affected dentin is similar to that from the naturally developed dentin caries lesions for both primary and permanent teeth.

CLINICAL SIGNIFICANCE

The pH-cycling model may be a suitable method to simulate caries-affected dentin in both permanent and primary teeth.

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