**Effect of dentin pre-treatment with Dolimite powder on demineralized dentine in deciduous molars**

**ABSTRACT**

The objective of this study was to evaluate mineral alterations and repercussions on mechanical properties in sound and demineralized dentin of primary molars after treatment with powdered dolomite (DMT) followed by glass ionomer cement (GIC) restoration. Class I cavities were prepared in 32 decidous molars, divided into groups G1 (sound dentin) and G2 (demineralized dentin). The 16-tooth (G1DMT, G2DMT) received topical application of DMT and restoration of high viscosity GIC . The 16 teeth assigned to the groups (G1 and G2) were restored with GIC. The specimens were sliced and prepared for Knoop (KHN), Micro Raman and FEG microhardness analysis. The variables ( sound and demineralized dentin), treatment (without and with DMT) and interaction (dentin and treatment) were analyzed with factorial ANOVA and Bonferroni post-test at a significance level of 5%. There was a significant difference in dentin interaction and treatment (p = 0.001). DMT associated with GIC determined improvement in the quality of the demineralized dentin substrate, with positive repercussions of the chemical-mechanical properties of the dentin.

Key words: Dental caries. Dentin. Glass Ionomer Cement.

**INTRODUCTION**

The current approach to a clinical situation of deep caries in deciduous teeth is the technique of partial removal of carious tissue (1), which consists of the removal of non-remineralizable carious tissue. The deeper layer of the cavity preparation (bottom wall) should not be removed to avoid pulpal exposure (2,3). Thus, in the clinical situation described, the therapeutic procedure of indirect pulp capping (IPC) is used. In that, the layer of infected dentin (superficial) with the presence of bacterial invasion, extensive demineralization, degradation of collagen fibers and infected dentin is removed (4). Indication of the technique should add to the absence of spontaneous pain, absence of mobility in the periodontal tissues or presence of radiopacity in the furcation zone or peri-apex and absence of pathological root resorptions (4). The IPC technique has been used for deciduous dentition. Clinical evaluations, radiographic and microbiological aspects of this treatment employed in carious lesions close to the pulp and at risk of exposing it have been made with very positive results.

The use of glass ionomer cements (GIC) is indicated for IPC due to its adhesion capacity to the dentin structure, coefficient of thermal expansion and modulus of elasticity similar to the tooth, besides the fluoride release and biocompatibility when applied on dentin (5,6,7). Another peculiar characteristic of GIC consists in the syneresis and inhbibition after its manipulation, which can result in hydrolytic degradation and consequently compromise the mechanical properties of the GIC (8,9,10). However, the search for materials that allow high rates of success for conservative pulp treatment is constant. When considering the repair process as a physiological process, the clinical challenge lies in the attempt to improve the condition of the carious dentin substrate. It means that the demineralized substratetso receives a restorative material that can promote greater longevity of the restorations.

The research for a biomaterial capable of improving the chemical and mechanical properties of the demineralized dental substrate from the chemical interaction with dentin was proposed in this study by including dolomite (DMT) to increase the amount of available calcium in the attempt to alkalize the demineralized and sealed in the technique of atraumatic restoration. The Brazilian Dolomite (Gran-White) is a common mineral composed of double carbonate of calcium and magnesium (CaMg (CO3) 2), a rock discovered by French geologist Deodat Dolmieu in the Tyrolean Alps (1750-1801) (11,12). In the chemical reaction it precipitates in the form of Ca and Mg carbonates, which can promote crystallization through a sedimentation mechanism of DMT (12). Its white color and very fine texture favors the absorption and fixation of calcium in the bones and the magnesium present in DMT activates more than 326 enzymatic systems of the human body, considered fundamental elements for the organismo (11,12). The formation of DMT, according to some studies, is capable of decreasing cultures of surface bactéria (10,12). Since the deposition of DMT crystals can undergo maturation and recrystallization together with increased crystallinity, which can produce a highly chemically ordered material (12,13). Moreover, the Mg/Ca rates increase carbonate precipitation (12). It is known that at balanced concentrations of fluoride (greater than 0.05 mM), from the sorption process, precipitation occurs in calcium fluoride through the release of Ca ions from CaO (14). The changes in the concentrations and pH of the F-1, Mg+2 and Ca+ 2 ions present in DMT decrease rapidly and remain in equilibrium after 24h(13,14). What is known through solid waste EDS models is that F sorption promotes the formation of CaF2. So that DMT can be a powerful adsorbent of fluorides. However, there are no clinical studies validating such effects (10-14), nor any applications in dentistry.

The study of the chemical exchanges between bioactive materials and dental tissues has been the subject of researches, as well as the changes in the physicochemical properties of the dental structures involved (15). Therefore, this in vitro study aimed to evaluate the chemical exchange of DMT applied prior to restoration of GIC, in deep dentin demineralized deciduous and repercussions on the mechanical-chemical properties of the dental tissues involved.

**MATERIAL AND METHODS**

This research was approved by the Ethics in Research Committee, protocol # 1.565.693 .

Sample

The sample consisted of 40 deciduous molars in order to obtain a higher standardization of the sample and also due to the difficulty in obtaining a group of sound deciduous molars. Eight teeth were lost during preparation of specimens. The sample consisted of 32 primary molars without any crown defects or carious lesions. The remaining root portions were removed using a high-speed diamond bur (2121 KG Sorensen®, Cotia, SP, Brazil), and the teeth were stored in distilled water at 37°C temperature to avoid dehydration until cavity preparation.

**Study Design**

Knoop hardness, Micro Raman and FEG tests were used to evaluate the following dentin conditions: sound/demineralized, with or without DMT aplication powder and with/without contact with GIC. For this purpose, class I cavities were prepared in the 32 selected primary molars. The teeth were divided into 2 groups (n=16) according to the dentin condition: sound (G1) and demineralized (G2). Subgroups (n=8) were formed to analyze the isolated action of the GIC and the action associated DMT powder.

The cavities were divided into halves, one of which was isolated with nail varnish, thus ensuring a control area in each group (Figure 1). All cavities were restored with a high-viscosity glass ionomer cement, but only G1DMT and G2DMT received a pretreatment with DMT powder before restoration (Figure 1).

**Preparation of the class I cavities**

A drilling apparatus (El Quip ®, São Carlos, SP, Brazil) was used in the preparation of the cavities, which were standardized (depth=2 mm, length=6 mm and wide=4 mm). The size of the cavities was checked with a caliper (Digimess®, São Paulo, SP, Brazil). The cavity size was based on a deep cavity, characteristic of deep carious lesions. Deep cavities are considered with more than 0,5mm and less than 1 mm remanescent structure between floor and pulp. The preparation of the cavities was started in the center of the occlusal surface   
of each primary molar. Tungsten carbide burs #8 with an initial depth of 1.5 mm were used to reach the final size set at 2mm with a diamond high rotation #3131, under continuous cooling. After 8 cavities, drills were replaced by new ones. To complete this step, the teeth were taken to the ultrasonic tank (Cristófoli Biosafety ®, Campo Mourão, PR, Brazil) for 280s to remove debris from the cavity preparation. Visual examination of specimens with a stereoscopic magnifying glass (10X) allowed verification of whether the pulp chamber was exposed by cavity preparation. If so, the tooth was discarded.

**Procedures for pH cycling**

The teeth of G2 (n = 16) were subjected to dentin demineralization to simulate carious lesions using the method of pH cycling according to the protocol already used11. To protect the external area of dental crowns, two layers of nail varnish (Colorama ®, Rio de Janeiro, RJ, Brazil) were applied, avoiding contact with the walls of the cavity.

To establish the DES-RE cycle, each specimen was immersed for 8 h in demineralizing solution and for 16 h in remineralizing solution. The volume of the demineralizing and remineralizing solution was 10 ml for each tooth in every cycle. This cycle was repeated for 14 consecutive days at room temperature without stirring. The solutions used in pH cycling were manipulated in the Pharmaceutical Science Lab   
at UEPG. The formulations (16) were as follows: demineralizing solution (pH 4.8) with 2.2 mM calcium chloride (CaCl2); 2.2 mM phosphate Sodium (Na2PO4) and 50 mM acetic acid; and remineralizing solution (pH 7.0): 1.5 mM calcium chloride (CaCl2), 0.9 mM phosphate Sodium (Na2PO4) and 0.15 mM potassium chloride (KCl).

**Application of DMT powder**

Before the procedures, all the cavities received a layer of nail varnish on the mesial side cavity. This research design stipulated the division of the cavity into two sites: control area (NC) and test area (C). This was done to guarantee that the GIC would not be in contact with the dentin, as well as to make sure that the DMT would be the only chemical element in contact with the dentin.

Application of DMT powder was carried out only for G1DMT and G2DMT (Figure 1) in the cavity fund. For these groups, after the application of the Ketac liquid and before the restoration, a pre-treatment with DMT was applied on the dentin for 1 min with the aid of a GIC dispenser, and the volume to each cavity was standardized. It was taken to cavity fund with the aid of a measuring spoon of GIC.

**Restorative Procedures**

All cavities were filled with high-viscosity GIC (Ketac Molar Easymix®, 3M ESPE, St. Paul, MN, USA). The cavities were treated with Ketac conditioner (3M ESPE, St. Paul, MN, USA) for 10s, washed with air/water spray for 20s, dried with a gentle stream of dry compressed air and immediately filled with the GIC. The GIC was dosed at a ratio of 1:1 (powder and liquid), and manipulated on the block by mixing with a plastic spatula (Duflex ®, Rio de Janeiro, RJ, Brazil). The mixture was inserted with an applicator syringe (Precision Maquira ®, Maringa, PR, Brazil) until the cavity was filled. The glass ionomer was allowed to set for 3 min, in accordance with the instructions of the manufacturer. Following restoration, the teeth were stored for 24 h at 37˚C.

**Preparation of specimens for tests**

The teeth were fixed in a cutting machine (Isomet ® 1000 Precision Saw Buehler, Lake Bluff, IL, USA) and were sectioned vertically with a diamond disk (1.3 mm Precision Saw, Lake Bluff IL ®, USA) at 300 rpm to obtain dental slices (n=3) with approximately 1.1 mm thickness.

Two dental slices were mounted in the center of PVC (Tiger ®, Joinville, SC, Brazil) cylinders (12x20 mm), which were attached with double-sided tape (3M®, SUMARE, SP, Brazil) on a glass plate. The cylinders were filled with colorless acrylic resin (JET ®, Clear Field, SP, Brazil) made by the powder and liquid technique

The embedded slices were taken to the rotary polishing machine (Arotec®, Cotia, SP, Brazil) to perform the polishing of specimens. A sequence of silicon carbide sandpapers (3M ® Brazil, Sumaré, SP, Brazil) was used under intense water irrigation. Final polishing was performed with diamond paste (Arotec®, Cotia, SP, Brasil) of grain 1/¼ µm. For removal of waste, the specimens were washed for 12 min in an ultrasonic tank. Finally, they were stored at 37˚C for 24 h in a 100% humidity environment.

**Microhardness Test**

The microhardness analysis was performed on a microhardness apparatus (Shimadzu®, Kyoto, Japan) with a Knoop indenter. For 30s, the applied loads were 25g for sound dentin and 10g for demineralized dentin(16).

The loads were applied on the specimens 50 µm below the cavity floor, wich characterized a deep cavity. Three different measures were made at 100 µm distance from each other at the same depth (16). The mean between these three measures was considered the microhardness value of the specimen.

**Evaluation of the ions in dentin by scanning electron microscope by field effect (FEG)**

For evaluation in FEG, five dental slices of each group were separated. The specimens previously selected were glued on metal stubs with a cyanoacrylate ester layer (Pegamil bondgel ultra forte, São José, SC, Brazil) with the aid of the microbrush. Afterwards, the specimens were stored in an oven for 48 h for drying, prior to reading.

The reading was performed at 50 μm depth of the cavity base with magnification of 5000x. The reading regions were divided into contact (C) and non-contact (NC) areas of the restorative material with dentin, a limit imposed by the presence of red enamel on cavity. Two types of FEG analysis are possible: EDS (Energy Dispersive Spectroscopy) and EBSD (Electron BackScatter Diffraction) through chemical and morphological characterization.

**Micro-Raman Spectroscopy**

The same specimens used for the microhardness test were subjected to analysis of mineral composition through micro-Raman spectroscopy (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). The apparatus was calibrated first to zero and then for the values ​​of coefficients using a sample of silicone.

The test included the following parameters: a neon laser with 532 nm wavelength and 20 mW, a spatial resolution of ≈ 3 mM, spectral resolution of ≈ 5 cm-1, 20 s accumulation time and 5 co-additions at 20X magnification (Olympus UK, London, UK) for a laser ≈ 1 mm in diameter. Readings were taken in a spectral range 300-1800 cm-1. The spectra were obtained from the dentin just below the tooth-restoration interface in a random place with a three-point mapping analysis: 0, 0.45 and 90 µm ​​in depth.

The micro-Raman spectroscopy detected the chemical content of the dentin through the vibrational molecular characteristics of energy. The representative spectra of calcium phosphate (corresponding to 960 cm-1) were identified, and peaks of different vibrational modes of the phosphate group (591 cm-1, 430 cm-1), carbonate (1070 cm -1), collagen (1270-1453cm-1) and peak and peak DMT were represented by the interval of 1080cm-1 present in the samples.

Statistical Analysis

The distribution of normality was verified with D'Agostino & Pearson and Shapiro Wilks tests; homogeneity of variances was tested with Levene's test. The hardness data were analyzed using ANOVA 2 criteria considering the factors dentin (sound and demineralized), treatment (with and without DMT) and dentin-treatment interaction. The level of significance used was 5%. The data micro-Raman spectra e FEG were analyzed qualitatively by groups. The statistical program GraphPad Prism version 5 for Windows (GraphPad Software, San Diego California, USA) was used for data analysis of hardness.

**RESULTS**

There was a significant difference in hardness values in DMT treatment in the NC and C areas (p <0.001); (Figure 2). There was a significant difference for the dentin factor in NC (p <0.0001). In the dentine – treatment interaction in NC (p = 0.238) there was no significant difference nor neither C (p = 0.391).

The data obtained in the Micro-Raman by spectroscopy are shown in the Figure 3. The range of the spectral region involved (275-1700 cm-1) was identified in all groups, with the highest intensity of the indicated peaks listed below: 1- 960 cm -1 (PO-3)4., 591 cm- 1 and 430 cm-1 (peaks of different vibrational modes of the phosphate group) representative of calcium hydroxyapatite, 2 -1070 cm -1 (carbonate), representative of the product formed by the CIV within the collagen fibers and 3 - 1080 cm -1 Peak of DMT found in G1 DMT and G2 DMT groups. In the Micro-Raman spectrum of the G1 DMT C group, within the range of the spectral region involved (275-1700 cm-1), the spectra of the phosphate group at absorbance in the range of 0-1500 were characterized. When analyzing the three depths (0, 45 and 90μm) relative to the cavity base, the highest absorbance peak was found in the cavity base. In the micro-Raman spectrum of group G1 GMT NC, within the range of the involved region (275-1700 cm -1) in the absorbance range (0-1500) there was difference in the analyzes of the three depths, being the level of Higher absorbance found in the cavity base. The DMT peak was not identified. In the micro-Raman spectrum of the G2 DMT C group within the range of the spectral region involved (275-1700 cm-1), three spectras were characterized at absorbance in the range of 0-1300. The phosphate peak was evidenced in two intervals, being 960cm-1 and 591cm-1. The highest peak of phosphate was found in the cavity base. DMT was identified at peak 1080 cm-1. In the micro-Raman spectrum of the G2 DMT SC group, within the range of the involved region (275-1700 cm-1) the depth 90μm obtained the highest peak at the 960 cm -1 phosphate peak.

**DISCUSSION**

The demineralized dentin obtained by artificial caries lesion created by the modified pH cycling method (16) attempts to simulate the mineral saturation dynamics associated with the pH variation of the natural caries formation process. This laboratory method is considered adequate to evaluate the loss and mineral gain of the dental substrate. Marquezan et al. (2009) (16) found in the specimens mineral loss with expressive reduction of hardness, reaching almost half of the value of the healthy dentin of deciduous teeth (34 KHN). Some studies have evaluated ion exchange values ​​between dentin and restorative material through artificial carious lesions and have concluded that the in vitro method allows detailed studies of the interactions between restorative material and demineralised dentin (15,16).

The mean values, according to a study employed by Marquezan et al. (2009) (16), in the test of hardness in the deciduous integral dentin vary from 50 to 70 KHN and the average values ​​in the demineralized dentin are below 50 KHN, generally around 30 KHN. In this research the demineralized dentin (G2 DMT) was the most reactive substrate, since there was a higher percentage increase of ions, both in SC and C, which suggests that DMT is a direct depositor of minerals that can contribute to the increase of the chemical properties of the Dentin substrate. Since mineral demineralized dentin occurs, both in the peritubular and intertubular dentin (17,18)

Complete removal of carious tissue compared to partial removal produces similar results in terms of dental caries progression and restoration longevity. Although there are high rates of clinical success reported in many studies, the uncertainty still lies in the maintenance of the affected dentin. However, partial removal of carious tissue has been commonly employed by pediatric dentistry (4,5,6). Among the characteristics that contribute to the increased success of pulpar treatment, in which the base resides in the maintenance of the remaining carious dentin, contributes to the technique consists of an alternative treatment for pulpotomy in the treatment of deep cavities without signs of pulpal degeneration (1,2). Regarding the liner products, calcium hydroxide is a successful technique, consisting of a sensitive method for deep cavity treatment in deciduous teeth due to the stimulation of the production of reactive dentin (19). Studies have shown that the clinical effectiveness of substances in residual dentin after partial removal of caries (19,18,10,4,3,1) promoves a longevity of restoration. Dentistry has moved from the surgical approach of caries lesions to the philosophical of minimal intervention to control and manage caries lesions, by preventing the cariogenic process from developing into symptoms. This should be evidenced by the application of any substances in the caries.

DMT is composed of calcium, which results in high values of this ion release (20,21,22). Another factor of importance is that studies have revealed that the composition of dentin, enamel and cement concentrate high values of Magnesium, which also present in the Dolomite could affect in the metabolism of mineralization, by increasing the Magnesium gradient. Furthermore, it reveals that, indirectly, magnesium would influence metabolism by enzymatic factors, directly due to effects of the crystallization process in the inorganic mineral phase (13,20,21,22). DMT would have action of a magnesium and calcium 'repository', in which it could cause an increase of calcium / phosphate ions in the receptor substrate. In studies, DMT was chosen as a repair material because of its composition rich in calcium and magnesium, important minerals in the constitution of the inorganic bone matrix (11,20,21,22). It suggests the stimulation of osteogenic action by playing a supporting role in the formation of new mineral complexes in contact with dentin. And data on the surface chemical composition of DMT, considered effective action of F adsorption and formation of CaF2(9,12,14). It coincides with the high values of calcium, phosphorous and fluoride minerals performed in this study through FEG as well as significant differences in the hardness tests in the region where DMT had isolated action of the GIC. Regarding the results of the Micro-Raman, the demineralized dentin in direct contact revealed the presence of Dolomite, proving its stability and alkalinization of the medium while promoting high indexes also of phosphate. In this way, more studies could be done to analyze the possibility of using DMT as a constituent of dental material, since this study revealed the effects of surface treatment of carious dentin.

The application of DMT to dentine prior to the application of GIC to the filling of the cavity demonstrated mineral gain with repercussion on the mechanical properties of the demineralized dentin in direct contact with the DMT, being verified a greater potential of mineral alteration when applied alone. However, the shortage of DMT information related to the field of dental material leads to limited knowledge of mechanisms of remineralization of caries lesion through the use of DMT. The information present in the literature is limited to the chemical and synthetic part of DMT, so that the reactivity should be better explored in relation to bioactivity, mainly due to the significant difference found in the demineralized dentin in direct contact with DMT. The present study used hardness data that provided data to elucidate the mechanical properties of deciduous teeth, together with the transformations of dentin chemical composition through FEG and Micro-Raman.

The data can predict the behavioral model of the dentin, DMT and GIC interaction. This research showed that mechanical properties of deciduous teeth at microscopic levels are highly dependent on the chemical and mechanical microstructural characteristics of the materials used in direct contact with the demineralized structure, capable of altering mineral composition and affect the improvement of the mechanical properties of the structures

**CONCLUSION**

From the results of this study, DMT could be an alternative material for deep carious lesions in deciduous teeth, due to changes in the chemical and mechanical properties of the demineralized deciduous dentin. However, it can not be considered a determinant factor for the success of restorative procedures, due to the incipient knowledge of DMT in dental products.

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