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The hardness and chemical changes in demineralized primary dentin treated by fluoride and glass ionomer cement

As mudanças químicas e de dureza na dentina decidua desmineralizada tratada com fluoreto e cimento de ionômero de vidro

Gisele Fernandes DIAS[a*], Ana Cláudia Rodrigues CHIBINSKI[a], Fábio André dos SANTOS[a], Viviane HASS[a], Fabiana Bucholdz Teixeira ALVES[a], Denise Stadler WAMBIER[a]

[a]UEPG – Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brasil

Resumo

Introdução: O fluoreto desempenha importante papel no controle da cárie dental. **Objetivo:** Avaliar as trocas químicas entre cimentos de ionômero de vidro de alta viscosidade (CIV) e dentina decídua com aplicação de fluoreto de sódio (NaF) a 2% em alterações de dureza dentinária a partir da incorporação de cálcio, fosfato e fluoreto. **Material e método:** Cavidades Classe I foram preparadas em 40 molares hígidos divididos em 2 grupos (n=20), de acordo com a condição dentinária: hígida (1) e desmineralizada (2). Subgrupos (n=10) foram formados para avaliar a ação isolada do CIV ou associado com NaF (F). Este estudo *in vitro* avaliou as trocas químicas sob duas condições: dentina hígida e desmineralizada (ciclagem de pH) para simular a perda mineral que ocorre em lesões de cárie. Grupo G1 e G2 receberam restaurações de CIV; Grupos G1F e G2F receberam NaF antes do CIV. Os espécimes foram preparados para microdureza Knoop e Micro-Raman. Para análise estatística foi utilizada Anova 2 fatores ($\alpha = 0.05$). Os dados do Micro-Raman foram descritos qualitativamente. **Resultado:** O aumento de dureza foi observado em todos os sítios de contato direto com CIV, em ambas dentinas em todos os grupos ($p<0.001$); não foi observado diferença em microdureza após aplicação do NaF ($p>0.05$). Na avaliação do Micro-Raman, o contato direto do CIV/dentina tanto hígida quanto desmineralizada resultou em um aumento do pico do fosfato dentinário. **Conclusão:** As trocas químicas entre o CIV e dentina desmineralizada podem induzir mudanças das propriedades mecânicas do substrato e a captação de íons minerais (fosfato) ocorre sem a influência do NaF.

Descritores: Cimentos de ionômero de vidro; dentina; tratamento restaurador atraumático; cárie dentária; fluoreto.

Abstract

Background: Fluoride plays an important role in the control of dental caries. **Aim:** To evaluate the chemical exchange between restoration of glass ionomer cement of high viscosity (GIC) and primary dentin with application of sodium fluoride (NaF) 2% through changes in hardness from uptake of calcium, phosphate and fluoride. **Material and method:** Class I cavities were prepared in 40 sound primary molars, and the sample was divided into two groups (n=20) according to dentin condition: sound (1) and demineralized (2). Sub-groups (n=10) were formed to investigate the isolated action of the GIC or the association with NaF (F). This in vitro study examined the chemical exchange under two conditions, sound and demineralized dentin (pH cycling), to simulate the occurrence of mineral loss for the caries lesion. G1 and G2 received GIC restoration only; groups G1F and G2F received NaF before GIC restoration. The specimens were prepared for Knoop hardness test and micro-Raman spectroscopy. A two-way ANOVA test ($\alpha = 0.05$) was used for statistical analysis. Micro-Raman data were qualitatively described. **Result:** Increased hardness was observed in all the sites of direct contact with GIC in sound and demineralized dentin for all groups ($p<0.001$); no difference was observed in microhardness after application of NaF ($p>0.05$). In the evaluation of micro-Raman, direct contact between GIC and dentin for sound and demineralized dentin resulted in increased peaks of phosphate. **Conclusion.** The exchange between GIC and demineralized dentin may induce changes of mechanical properties of the substrate, and uptake of mineral ions (phosphate) occurs without the influence of NaF.

Descriptors: Glass ionomer cements; dentin; atraumatic restorative treatment; tooth decay; fluoride.

INTRODUCTION

The contemporary approach to restorative procedures in pediatric dentistry is based in minimally invasive dentistry. It favors partial caries removal and the maintenance of carious dentin, which still has the potential for reorganization. In primary teeth, clinical researches have already proved this potential after restoration using glass ionomer cements[1-3] (GIC) or other restorative materials[4-6]. Nevertheless, there is a trend in research toward study of the interaction between demineralized dentin and bioactive materials such as glass ionomer cements. When using GIC, there is some ion exchange between ions that are released during the setting reaction of the material and the dentin minerals. This reaction allows not only the mineral gain of dentin[7] but also a more effective adhesion that affects the longevity of the restoration.

The main ions involved in this process are calcium, strontium, aluminum and fluoride[7]. In particular, fluoride has always been a subject of research because of its participation in DES-RE mechanism[8] and because GIC's property of fluoride release and reload makes this material a reservoir of fluoride in the mouth.

The topical application of fluoride in the surface of enamel/dentin produces a calcium fluoride (CaF_2) layer with a high concentration of ionic fluoride, which controls dental solubility in situations of high cariogenic challenge[9]. Notwithstanding, there is little information available about the action of fluoride in demineralized[10] and sealed dentin as considered in this paper.

We hypothesized that the chemical exchange between fluoride solution and demineralized dentin, along with restoration of the glass ionomer cement, would improve the mechanical properties of the dentin. As a result, the adhesion and longevity of the restoration could be positively influenced.

Therefore, this paper's objective was to evaluate the chemical exchange between high-viscosity glass ionomer cement restoration and primary demineralized dentin with the application of sodium fluoride (NaF) 2% through changes in dentin hardness from uptake of calcium, phosphate and fluoride.

MATERIAL AND METHOD

This research was approved by the Ethics in Research Committee, protocol #134.145.

Sample

The sample consisted of 40 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 room temperature to avoid dehydration until cavity preparation.

Study design

Knoop hardness and micro-Raman tests were used to evaluate the following dentin conditions: sound/demineralized, treated or not treated with NaF solution and with/without contact with GIC. For this purpose, class I cavities were prepared in the 40 selected primary molars. The teeth were divided into 2 groups (n=20) according to the dentin condition: sound (G1) and demineralized (G2). Subgroups (n=10) were formed to analyze the isolated action of the GIC and the action associated with sodium fluoride (F).

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 G1F and G2F received a neutral solution of NaF 2% 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 preparation of the cavities was started in the center of the occlusal surface of each primary molar. Tungsten carbide

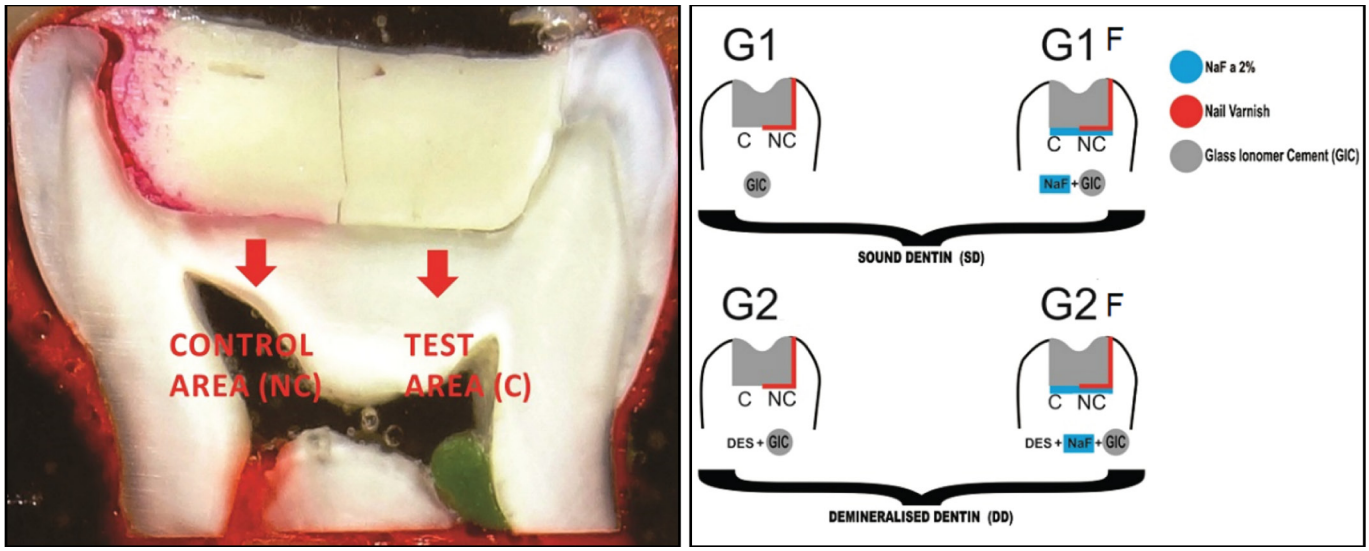

Figure 1. Sagittal section of teeth restored with GIC in two dentin conditions: sound/ demineralized (G1, G2) and with topical application of fluoride (NaF) before restoration (G1F, G2F). Red nail varnish layer has been applied to avoid direct contact of GIC with the half of the dentin - control area (NC) and test area (C).

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 280 s to remove debris from the cavity preparation. Visual examination of specimens with a stereoscopic magnifying glass (10×) 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=20) were subjected to dentin demineralization to simulate carious lesions using the method of pH cycling according to the protocol already used[11]. 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[12] were as follows: demineralizing solution (pH 4.8) with 2.2 mM calcium chloride ($CaCl_2$); 2.2 mM phosphate Sodium (Na_2PO_4) and 50 mM acetic acid; and remineralizing solution (pH 7.0): 1.5 mM calcium chloride ($CaCl_2$), 0.9 mM phosphate Sodium (Na_2PO_4) and 0.15 mM potassium chloride (KCl).

The restorative procedures were carried out right after the DES-RE cycle was finished for all the specimens.

Restorative Procedures

All cavities were filled with high-viscosity GIC (Ketac Molar Easymix®, 3M ESPE, St. Paul, MN, USA).

The protocol used is described above. The cavities were treated with Ketac liquid (3M ESPE, St. Paul, MN, USA) for 10 s, washed with air/water spray for 20 s, dried with a gentle stream of dry compressed air and immediately filled with the GIC. The GIC was dosed at a ratio of 2:2 (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, followed by the "press finger" technique (30 s) with polyester tape (K-Dent Quimidrol ®, Joinville, SC, Brazil). The glass ionomer was allowed to set for 3 min, then protected with a layer of petroleum jelly (Rioquímica®, São José do Rio Preto, SP, Brasil) in accordance with the instructions of the manufacturer. Following restoration, the teeth were stored in a humidifier for 24 h at 37 °C.

Topical Application of Neutral Solution of NaF

Topical application of neutral solution of NaF was carried out only for G1F and G2F (Figure 1). For these groups, after the application of the Ketac liquid and before the restoration, a neutral solution of NaF at 2% was applied on the dentin for 1 min with the aid of a cotton ball, and the volume to each cavity was standardized with a cotton ball. The excess solution was removed with filter paper discs.

Before the restorative procedures, all the cavities received a layer of nail varnish on the mesial side of the cavity. The 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 NaF solution would be the only chemical element in contact with the dentin.

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 (12×20 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 30 s, the applied loads were 25 g for sound dentin and 10g for demineralized dentin[12].

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

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 20× 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[12]. 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 (1270-1453 cm^{-1}) and peak CaF_2 were represented by the interval of 322 cm^{-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 fluoride) and treating dentin interaction. The level of significance used was 5% ($\alpha = 0.05$). The data micro-Raman spectra 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.

RESULT

The means and standard deviations from Knoop microhardness are displayed on Table 1. Here, we can note that there is an increase in Knoop hardness (KHN) in different groups. There was a great percentage increase of Knoop hardness for Group 2F, which revealed great uptake of fluoride by the demineralized dentin. Enhanced hardness values were observed in the test area in sound and demineralized dentin when compared to the control area ($p<0.0001$). There was a significant difference between the control area (NC) and the test area (C) in all groups assessed (Table 1).

A significant difference in Knoop hardness was found for dentin (sound and demineralized) in the control area ($p<0.0001$) and the test area with the restorative material ($p<0.0001$). Changes in hardness occurred because of direct contact of the restorative material with the dentin surface in both conditions. Treatment with NaF did not result in a significant difference for the dentin (sound and demineralized) in either the control area ($p=0.358$) or the test area ($p=0.642$). The interaction dentin x NaF did not effect significant change in the hardness of dentin, and there was no significant difference in either the control area ($p=0.309$) or the test area ($p=0.751$). Pre-treatment with NaF was not effective in improving the condition of the dentin (Figure 2).

The data obtained by micro-Raman spectroscopy are shown in Figure 3. The range of the spectral region involved (300-1700 cm^{-1}) was identified in all groups through the largest intensity peak indicated: 960 cm^{-1} (PO_4^{-3}- phosphate), 591 cm^{-1} and 430 cm^{-1} (peak vibrational modes of the phosphate group), which represent the calcium hydroxyapatite. The peak 1070 cm^{-1} (carbonate) represents the product of collagen fibers within the GIC[12]. The peak of 1270-1453 cm^{-1} characterizes collagen in the range, and 322 cm^{-1} is CaF_2. The identification of the peak of CaF_2 was sought in G2B and G1B, as the product formed after topical application of fluoride, whose reaction with fluoride apatite is the product of precipitation of fluoride ions[13].

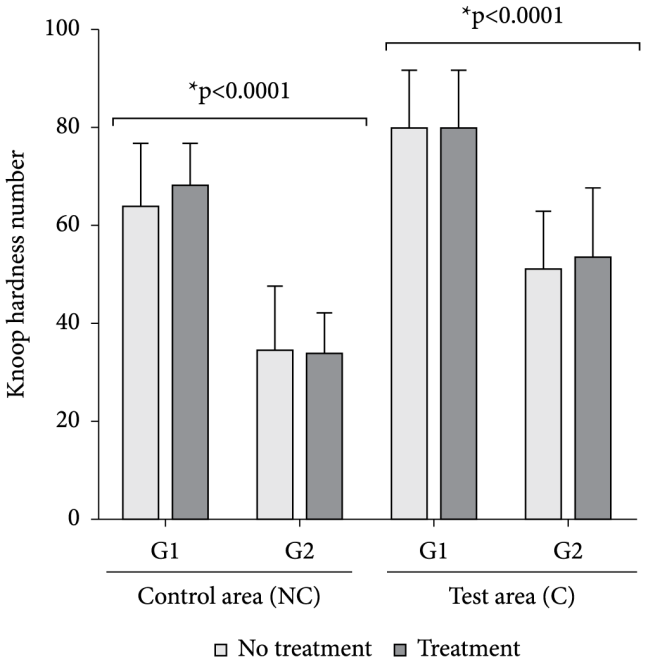

Figure 2. Mean and standard deviation of the Knoop hardness values. (*) Significant factor for dentin areas control and test area difference. Factor treatment (untreated and treated) no significant difference according to the control area ($p=0.358$) and test area ($p=0.642$). Interaction (factor dentin and treatment), no significant difference according to the control area ($p=0.309$) and test area ($p=0.751$). ANOVA with two criteria.

Table 1. Means, standard deviations and percentage increase in Knoop hardness (KHN) in different groups

Groups	Area		
	Control Area (NC)	Test Area (C)	Increase in Knoop hardness (%)
G1	63.3 ± 13.4	79.8 ± 11.9	21
G1 F	68.2 ± 8.5	80.2 ± 11.6	15
G2	34.1 ± 13.5	51.2 ± 11.4	33
G2 F	33.9 ± 8.5	53.4 ± 14.0	36

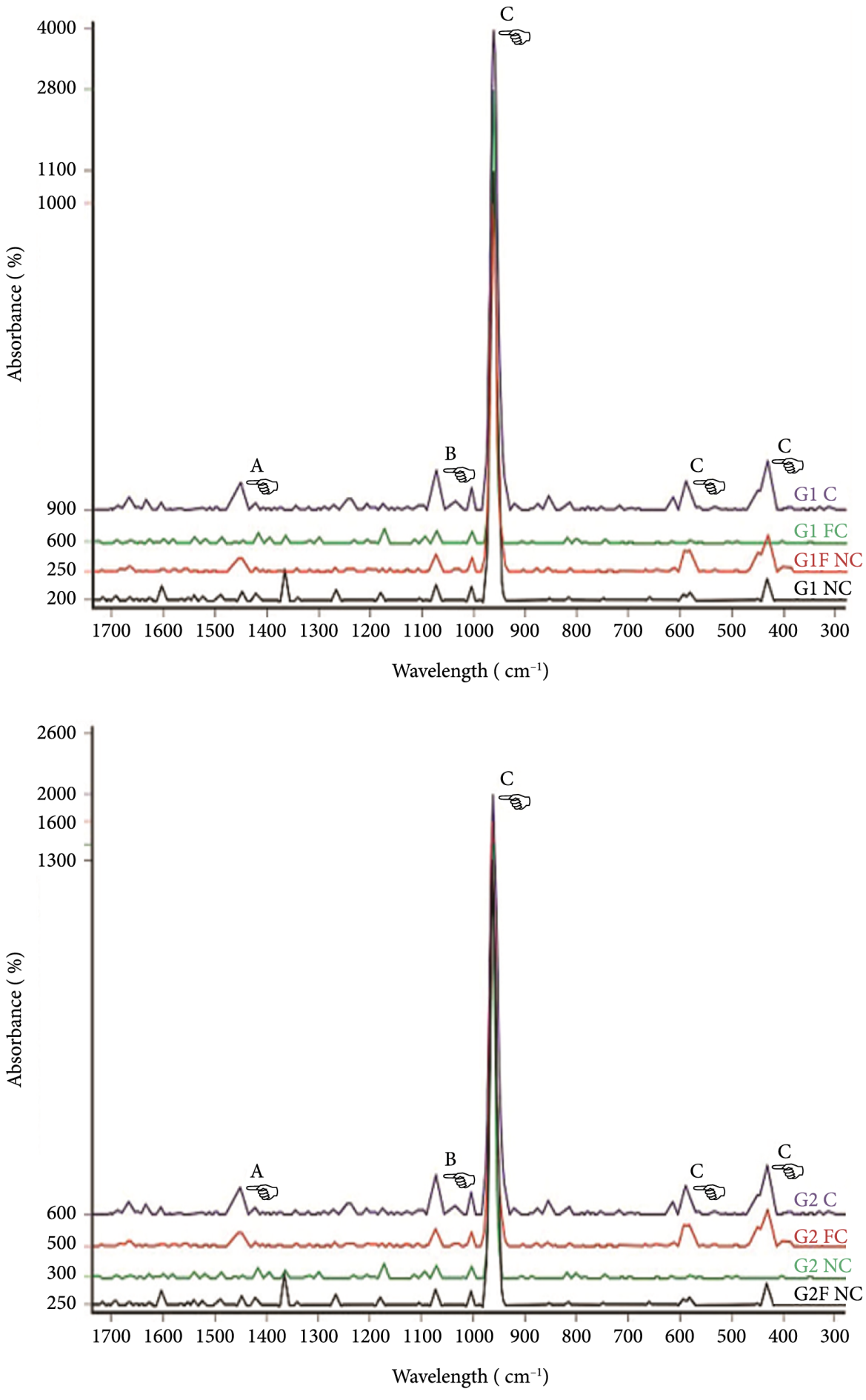

Figure 3. Spectra obtained from the analysis of the dentin composition by Micro-Raman. Control area (NC) and Test Area (C). The arrows indicate: (A) collagen; (B) carbonate; (C) phosphate.

DISCUSSION

We based our research in an *in vitro* model previously presented in the literature[10] to study ionic exchanges between demineralized dentin and GIC. As such, the present paper evaluated changes in the mineralization of dentin from uptake of calcium, phosphate and fluoride in demineralized primary dentin after GIC restorations, with or without application of a neutral solution of NaF 2%. In this paper, we observed that direct contact between dentin/GIC resulted in increased hardness and phosphate concentration in dentin substrate.

The increased hardness of demineralized dentin suggests an improvement[4,10,13] that is compatible with the changes in mineral composition. It is important to point out that these alterations weren't dependent of the fluorides in the GIC or the solution that was applied before tooth restoration, since we did not detect an increase in the fluoride levels in sound or demineralized dentin. This contradicts our hyphothesis, since we believed that when conditioning the dentin with a soft acid like the Ketac liquid, it would be enough to potentialize the action of a neutral fluoride solution. The fluoride in neutral solution probably wasn't reactive enough to form a stable mineral layer or to penetrate the dentin substrate. On the other hand, the fluoride released by the GIC wasn't incorporated on the dentin either. Therefore, we can say, based on our findings, that the improved mechanical property seen in the dentin substrate was dependent on the phosphate ions only.

Therefore, this result may be due to the ionic exchange in the tooth-restoration interface[13,14]. Ionic elements like sodium, calcium, aluminum and phosphorus migrate to demineralized dentin[13,15,16]. This mineral uptake promotes changes in the hardness of dentin, as we demonstrate in our paper. The relationship between hardness and mineral content of dental hard tissues is well documented in the literature[17,18].

The method used in our study produces artificial caries lesions based on a pH cycle model[11] that simulates the DES-RE mechanism and the pH variation associated with the carious process. The mineral loss resulting from this method translated into a significant reduction in Knoop hardness for the demineralized dentin, which reached a maximum of half of the values from sound dentin.

These modifications were evident in the micro-Raman analysis. We could identify the representative peaks from the mineral phase of the dentin and from apatite hidroxicarbonate, which is the product of the reaction between GIC and dentin[19,20], as well as the phosphate peaks in the sites that have direct contact with GIC.

The research design stipulated the division of the cavity into two sites: control area (NC) and test area (C). This was done in an attempt to minimize bias when comparing the dentin substrate with treatment or not. As such, we compared samples of dentin from the same tooth and at the same depth, and inherent variations in mineral content between different teeth did not influence the results.

Fluoride application before GIC restoration did not increase the fluoride uptake in the dentin. One possible explanation is that a lower pH environment should be achieved in the dentin/GIC interface in order for an additional fluoride solution to make some difference in the ion exchange proccess[21].

There are reports that show that the pattern of fluoride and strontium penetration in dentin after GIC restoration is consistent with the mineralization process[22]. Even so there is no evidence that ultrastructural remineralization occurs in the intra or interfibrillar demineralized collagen matrix[23]. Hence, the term "remineralization" isn't being used correctly. As a matter of fact, GIC promotes a "mineral gain" in the demineralized dentin, with enhanced calcium and phosphorus contents[4,16], but it cannot be considered remineralization[23].

Chemical analysis using micro-Raman spectroscopy complemented the characterization of the dentin underlying the GIC restoration. It provided microscopic data for a wide swath of specimens through vibrational molelular alterations[24,25]. We were able to detect the peak of 960 cm^{-1}, which represents phosphate, in all tested groups. This is the most intense micro-Raman peak in dental hard tissues which indicates mineral accretion in dentin.

In this study, the methodology allowed us to assess the isolated action of the GIC, thus the microhardness test was effective. Gains in hardness and changes in mineral composition may induce immediate improvement in the condition of dentin[4,10,13], and no changes were observed with application of a neutral solution of NaF 2%. Therefore, the results reinforce the importance of GIC as a "therapeutic" and firstchoice material for minimally invasive restorations, especially in the ART strategy.

CONCLUSION

A neutral solution of NaF 2% did not modify the mineral and mechanical characteristics of sound and demineralized dentin. The increase in dentin hardness and changes in mineral content were due to ion exchange from GIC to dentin.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

*CORRESPONDING AUTHOR

Gisele Fernandes Dias, Departamento de Odontologia, UEPG – Universidade Estadual de Ponta Grossa, Rua Coronel Francisco Ribas, 396, apto 4, Centro, Ponta Grossa - PR, Brasil, e-mail: giodonto@hotmail.com

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