

**UNIVERSIDADE DE SÃO PAULO  
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO**

**SILVIA JORGE DOMICIANO**

**MICRODUREZA DA DENTINA RADICULAR ADJACENTE A MATERIAIS RESTAURADORES  
CONTENDO FLÚOR APÓS DESAFIOS EROSIVOS: ESTUDO *IN SITU* / *EX VIVO***

**RIBEIRÃO PRETO  
2007**

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CONTENDO FLÚOR APÓS DESAFIOS EROSIVOS: ESTUDO *IN SITU* / *EX VIVO***

Dissertação apresentada à Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo para obtenção do título de mestre em Odontologia Restauradora.  
Área de concentração: Dentística  
Orientadora: Profa. Dra. Mônica Campos Serra.

**RIBEIRÃO PRETO  
2007**



## FOLHA DE APROVAÇÃO

Silvia Jorge Domiciano.

Microdureza da dentina adjacente a materiais restauradores contendo flúor após desafios erosivos: estudo *in situ/ ex vivo*.

Dissertação apresentada à Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo para obtenção do título de mestre em Odontologia Restauradora.

Área de concentração: Dentística

Aprovado em: \_\_\_/\_\_\_/\_\_\_.

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Prof. Dr. \_\_\_\_\_

Instituição: \_\_\_\_\_ Assinatura: \_\_\_\_\_

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Prof. Dr. \_\_\_\_\_

Instituição: \_\_\_\_\_ Assinatura: \_\_\_\_\_

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**À Deus**, por seu infinito amor e grandeza, que me presenteou com a vida.

**Aos meus pais, Hélio e Terezinha**, por seu amor incondicional, ajudando, amparando e apoiando em todos os momentos, sorrindo a cada sorriso meu e enxugando cada lágrima derramada. Vocês foram e são minha base, possibilitando a realização de todos os meus sonhos, conduzindo meus passos, para trilhar uma vida repleta de conquistas e felicidade. A palavra desistir nunca existiu para mim graças a imensa confiança de vocês em minha capacidade. Obrigada Papai e obrigada Mamãe.

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*“Bom é louvar o Senhor, e cantar salmos ao teu nome, ó Altíssimo”:  
anunciar pela manhã a sua misericórdia, e a tua fidelidade durante a noite,  
com o saltério de dez cordas, e a lira, com cântico ao som da cítara.*

*Por que me alegras, Senhor, com as tuas obras, e eu exulto  
com as obras das tuas mãos.*

*Quão magníficas são, Senhor, as tuas obras! Quão profundos  
são os teus pensamentos!*

*O homem insensato não conhece, e o néscio não compreende estas coisas.  
Embora os ímpios floresçam como a erva, e brilhem todos os que fazem mal,  
estão destinados a eterno extermínio;*

*Mas tu, Senhor, és eternamente excelso.*

*Pois eis que os teus inimigos, Senhor, eis que os teus inimigos perecerão, e  
serão dispersados todos os que praticam o mal.*

*Exaltastes a minha força como a de um búfalo;  
ungiste-me com azeite puríssimo.*

*E os meus olhos olharam com desprezo para meus inimigos, e os meus  
ouvidos ouviram alegres novas dos malignos que se levantaram contra mim.*

*O justo florescerá como a palma, e como o cedro do Líbano crescerá.  
Plantados (os justos) na casa do Senhor, florescerão nos átrios do nosso Deus.*

*Darão frutos mesmo na velhice, estarão cheios de seiva e de vigor,  
Para anunciar quão reto é o Senhor, minha Rocha,  
e que não há nele iniquidade “*

**Salmo 91.**

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*"Quem for fundamentalmente um mestre,  
apenas toma a sério tudo o que se relaciona com  
os seus discípulos, incluindo a si próprio."*

Friedrich Nietzsche

## SUMÁRIO

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## RESUMO

DOMICIANO, S. J.; COLUCCI, V.; SERRA, M. C. **Microdureza da Dentina Radicular Adjacente a Materiais Restauradores Contendo Flúor Após Desafios Erosivos: Estudo *in situ lex vivo***. 2007. 29p. Dissertação (mestrado) – Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2007.

Este estudo foi realizado a fim de avaliar a microdureza da dentina radicular adjacente a materiais restauradores contendo fluoretos após desafios erosivos. Foi utilizado um delineamento *crossover* de duas fases de 4 dias consecutivos cada. Cento e doze fragmentos de dentina bovina foram obtidos, nos quais foi confeccionada uma cavidade padronizada no centro de cada um. Estas cavidades foram restauradas aleatoriamente com cimento de ionômero de vidro (Ketac-fil) ou resina composta (Filtek Z-250). Em seguida, os fragmentos restaurados foram distribuídos aleatoriamente entre os 14 voluntários, montados em aparelhos, cada um com 4 espécimes restaurados com o mesmo material. A partir do segundo dia, metade do aparelho, com dois espécimes, foi imersa em refrigerante de limão por 90 s, quatro vezes por dia, fora da boca por 3 dias. Após um intervalo de 3 dias, fragmentos restaurados com o material ainda não utilizado foram montados no aparelho e foi iniciada a segunda fase do experimento. Após os desafios erosivos, foram realizadas as medidas da microdureza da dentina adjacente às restaurações. Independente do material empregado, os espécimes erodidos apresentaram menores valores de microdureza ( $p < 0.0001$ ). Com relação aos materiais restauradores, foi verificado que a dentina, nas condições deste estudo, apresentou maiores valores de microdureza quando restaurada com cimento de ionômero de vidro ( $p < 0.0001$ ). Sendo assim, podemos concluir que o cimento de ionômero de vidro diminui a progressão da erosão da dentina radicular adjacente à restauração.

**Palavras-chave:** erosão, dentina, material restaurador, flúor.

## **ABSTRACT**

DOMICIANO, S. J.; COLUCCI, V.; SERRA, M. C. **Effect of different restorative materials on root dentine erosion: an in situ/ex-vivo study**. 2007. 29p. Dissertation (master's degree) – School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, 2007.

This study sought to evaluate the microhardness of root dentine adjacent to fluoride-containing restorative materials after erosive challenge. A cross-over study was performed in two phases of 4 consecutive days each. One hundred and twelve bovine root dentine slabs were obtained, and standardized box-shaped cavities were prepared at center of each specimen. The prepared cavities were randomly restored with glass-ionomer cement or composite resin. The slabs were randomly assigned among 14 volunteers, which wore intraoral palatal device containing 4 restored root dentin slabs. Starting on the second day, half of the palatal acrylic devices were immersed extraorally in a lemonade-like carbonated soft drink for 90 seconds, four times daily for 3 days. After 3-day wash-out, dentine slabs restored with the alternative material were placed into palatal appliance and the volunteers started the second phase of this study. After erosive challenges, microhardness measurements were performed. Regardless of the restorative material employed, eroded specimens demonstrated lower microhardness value ( $p < 0.0001$ ). At any given dentine condition examined in this study, dentine restored with glass-ionomer cement showed higher microhardness values ( $p < 0.0001$ ). It may be concluded that the glass-ionomer cement decreases the progression of root dentine erosion at restoration margin.

**Key words:** erosion, dentine, restorative materials, fluoride.

## FICHA CATALOGRÁFICA

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## **INTRODUÇÃO**

---

Modelos de pesquisa *in situ* têm sido utilizados para avaliar as propriedades cariostáticas de materiais fluoretados (Ten Cate, J. M, 1994). Este tipo de experimento é considerado um estágio intermediário entre estudos *in vivo* e *in vitro* (Classen, A.B.S, Øgaard, B, 1999) e é útil nas pesquisas sobre erosão (Hunter, M. L. *et al*, 2003). Esse tipo de desgaste dental tem etiopatogenia relacionada à desmineralização dental (Lussi, A *et al*, 1995; Lussi, A *et al*, 2004), proporcionada pela ação de ácidos de origem intrínseca ou extrínseca na superfície dental sem biofilme (Ganss, C, 2006). De origem extrínseca, o consumo freqüente de ácidos de origem alimentar, como os refrigerantes (Hooper, S. M. *et al*, 2007), está associado à presença e progressão do desgaste erosivo (Lussi, A, 2006). Diversas estratégias têm sido descritas visando à redução do desgaste erosivo. Dentro desse contexto, a principal recomendação é reduzir a exposição a ácidos (Hughes, J. A *et al*, 2004; Lussi, A. *et al*, 2004, Lussi, A *et al*, 2006), através da diminuição da freqüência de ingestão e tempo de contato com a estrutura dental (Hughes, J. A *et al*, 2004; Lussi, A. *et al*, 2004). Entretanto, há casos em que a perda significativa de tecido dental, a hipersensibilidade e o envolvimento estético, requerem a instituição de medidas terapêuticas restauradoras, para devolver a forma e a saúde (Lambrechts, P. *et al*, 1996; Levitch, L.C *et al*, 1994).

As alterações no conteúdo mineral provocadas pela exposição a bebidas ácidas podem interferir na dureza do tecido dentinário. Medidas de microdureza superficial permitem a detecção de estágios iniciais de enfraquecimento da dentina desmineralizada (Attin, T, 2006). A utilização de material fluoretado na restauração de lesões de erosão também poderia influenciar esta dinâmica, dependendo da concentração deste íon. Dentre os materiais utilizados destacam-se os cimentos de ionômero de vidro convencionais e, as resinas compostas. Em relação à progressão de lesões de cárie, há evidências laboratoriais de que os cimentos de ionômero de vidro previnem e/ou controlam a formação destas lesões em esmalte na cavidade e também a uma distância da restauração (Hara, A. T, 2002). Entretanto, o efeito desse

material na dentina radicular, frente a desafios erosivos, em ambiente bucal, ainda não foi verificado.

Sendo assim, este estudo avaliou a microdureza da dentina radicular adjacente ao material restaurador contendo fluoretos quando submetidos a desafios ácidos, através de um modelo *in situ*.

**PROPOSIÇÃO**

---

Essa dissertação tem como objetivo apresentar um artigo científico para avaliar a influência de material restaurador com flúor na microdureza superficial da dentina radicular bovina adjacente a restaurações quando submetidos a desafios erosivos, *in situ/ ex vivo*.

**CAPÍTULO**

---

## **Effect of different restorative materials on root dentine erosion**

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**Abstract**

This study sought to evaluate the microhardness of root dentine adjacent to fluoride-containing restorative materials after erosive challenge. A cross-over study was performed in two phases of 4 consecutive days each. One hundred and twelve bovine root dentine slabs were obtained, and standardized box-shaped cavities were prepared at center of each specimen. The prepared cavities were randomly restored with glass-ionomer cement or composite resin. The slabs were randomly assigned among 14 volunteers, which wore intraoral palatal device containing 4 restored root dentin slabs. Starting on the second day, half of the palatal acrylic devices were immersed extraorally in a lemonade-like carbonated soft drink for 90 seconds, four times daily for 3 days. After 3-day wash-out, dentine slabs restored with the alternative material were placed into palatal appliance and the volunteers started the second phase of this study. After erosive challenges, microhardness measurements were performed. Regardless of the restorative material employed, eroded specimens demonstrated lower microhardness value ( $p < 0.0001$ ). At any given dentine condition examined in this study, dentine restored with glass-ionomer cement showed higher microhardness values ( $p < 0.0001$ ). It may be concluded that the glass-ionomer cement decreases the progression of root dentine erosion at restoration margin.

Key words: erosion, dentine, restorative materials, fluoride

## Introduction

Since 19<sup>th</sup> century, the incidence and prevalence of dental erosion have been increasingly being reported, mainly in adult patients.<sup>1</sup> Due to a chemical process, the contact of the dental surface with non-bacterial acids<sup>2,3</sup> promotes irreversible loss of dental tissue that leads to the formation of non-cariious lesions, such as dental erosion. This pathology can be caused by intrinsic agents, such as recurrent vomiting or regurgitation of the gastric content<sup>4,5</sup> or extrinsic factors, including the ingestion of acid drinks, foods or medication,<sup>6,7,8</sup> with the former being the major cause of the erosion.<sup>9</sup>

An accurate diagnosis becomes essential for an effective control of dental erosion. For extrinsic acids, the goal is to reduce acid exposure by decreasing the frequency of ingestion of potentially harmful drinks and foodstuffs as well as minimizing contact time.<sup>10</sup> However, owing to the restricted contribution of the patients in modifying their dietary habits, the damages caused by acid challenge can reach a certain degree, in which the aesthetic appearance is unacceptable<sup>11</sup> and dentine is hypersensitive<sup>12</sup> and thus, an oral rehabilitation becomes necessary.<sup>13</sup>

The restorative treatments should be as conservative as possible.<sup>14</sup> As result of the improvements in mechanical properties of filling materials and in adhesive techniques,<sup>13,15</sup> it has been possible to rehabilitate eroded dentition in a less-invasive manner.<sup>13</sup> The use of fluoride-releasing restorative materials, such as the glass-ionomer cement, has been associated with a cariostatic effect on root dentine subjected to cariogenic challenges,<sup>16,17,18</sup> by decreasing demineralization and enhancing remineralization process.<sup>19</sup>

*In vitro* and *in situ* studies have demonstrated that establishment of fluoridation measurements significantly reduce the erosion progression in enamel and dentine.<sup>20, 21</sup> Additionally, it has been shown that the use of slow-release devices for fluoride delivery may facilitate rapid remineralization enamel and dentine submitted to acid challenges.<sup>22</sup> However, there are no studies that evaluate the effect of fluoride from glass-ionomer cement restorations on progression of root dentine erosion.

Considering the limitations in literature, the current study sought to evaluate *in situ* the effect of fluoride-containing materials on progression of root dentine erosion.

## **Materials and methods**

### **Experimental Design**

A cross-over and double-blind study was conducted in two phases of four days each, with a 3-day wash-out period interposed at the cross-over point (Figure 1). Initially, the protocol for this study was reviewed and approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto (USP). Fourteen adult volunteers were selected and they received verbal and written information concerning the study and gave signed and witnessed consent to participate.

The factors involved in this study were restorative material in two levels: Group A, glass-ionomer cement (Ketac Fil, 3M Dental Products, St Paul, MN, USA); and Group B, composite resin - control (Filtek Z250, 3M Dental Products, St Paul, MN, USA); and dentine condition in two levels (I - eroded and II – uneroded). The experimental sample, comprised of 112 specimens of dentine,

was randomly assigned in replicate to the 14 volunteers, which were considered as statistical blocks. The response variable was microhardness measurements performed in dentine around the restorations.

#### *Specimen preparation and selection of slabs*

Bovine incisors, freshly extracted, with age standardized at four years, were cleaned with scaler and water/pumice slurry in dental prophylactic cups and they were stored in a 0.1% thymol solution at 4°C. Teeth were sectioned with a water-cooled diamond saw in a sectioning machine (Isomet 1000; Buehler, Lake Bluff, IL, USA) to obtain two fragment from the cervical third (3 x 3 x 2 mm), totaling 150 samples. The dentine surfaces were flattened and ground in a water-cooled polishing machine (Politriz DP-9U2, Struers A/S, Copenhagen, DK-2610, Denmark) with 400-, 600- and 1200- grades of Al<sub>2</sub>O<sub>3</sub> papers, and polished on cloths with a 0.3 µm alumina suspension (Buehler, Lake Bluff, IL, USA).<sup>23</sup> Slabs were immersed in deionized water and sonicated for 10 min to clear the surface. Afterwards, specimens were sterilized with ethylene oxide,<sup>23</sup> carefully inspected for surface defects, and were kept in 100% humidity environmental at 4°C.

Slabs were pretested for Knoop microhardness, in order to standardize the test pieces.<sup>23</sup> A total of 112 out of 150 slabs were selected based on the average SMH data.

#### *Cavity preparation and restoration of root dentine blocks*

Box-shaped cavities (2 x 2 x 1 mm) were prepared with #2096 diamond burs (KG Soresen, Barueri, SP, Brazil) at the center of each slab, with a high-speed handpiece (Dabi-Atlante, Ribeirão Preto, SP, Brazil). The high-speed handpiece was fixed in a milling machine (MPC, ElEquip, São Carlos, SP,

Brazil), in which axles movement were monitored by digital comparing clocks, supplying precision of 0.01 mm in the cavities dimension. New burs were replaced after every five preparations.

After cavity preparation, blocks were randomly assigned to be restored with one of the materials to be tested: Z250 (control) or Ketac Fil. The restorative procedures were performed according to manufacturer's instructions. For Z250, surfaces were treated with a 35% phosphoric acid gel (Scotchbond gel, 3M Dental Products, St Paul, MN, USA) for 15 seconds, rinsed for the same time and gently dried to keep a moist surface. Then, an etch and rinse adhesive system (Single Bond 2 Adper, 3M Dental Products, St Paul, MN, USA) was applied for 15 seconds; the remaining solvent was evaporated with a brief, gentle dry air jet and light-cured for 10 seconds. After that, a hybrid composite resin restoration was built on the cavity. The composite restoration was cured for 20 seconds, with a visible light-curing unit (XL 3000, 3M/ESPE, USA) with a 450-mW/cm<sup>2</sup> output. The restorative material inserted in the cavities was pressed for 1 minute with a glass-plate under a load of 500 g. For glass-ionomer cement restorations were allowed to cure for 7 min. Cavities restored with Ketac Fil were protected using non-colored nail varnish. After 24 hours in relative humidity at 37°C, the specimens were polished in a water-cooled polishing machine with 600- and 1200- grades of Al<sub>2</sub>O<sub>3</sub> papers and they were kept in 100% humidity environmental at 37°C.

#### Palatal appliance mounting

For each volunteer, one removable intraoral appliance was constructed in acrylic resin, with four sites (3 x 3 x 3 mm), being two in each side of the midline. Four dentine blocks restored with the same material were randomly

mounted into sites, with the restored surface recessed approximately 1.0 mm below the surface of the appliance to avoid tongue friction.<sup>23</sup> At the beginning and end of each experimental day, appliances with the contained specimens, were soaked in a 0.2% chlorhexidine mouthwash for 2 min. Overnight the appliances were placed in sealed containers on moist cotton wool.<sup>24</sup>

### Clinical phase

Before starting the intraoral procedures, a three-day *lead-in* period was established. The volunteers were instructed to keep habitual methods of brushing teeth and to use exclusively the toothbrush and dentifrice supplied to them by the researchers. All volunteers employed a fluoride-containing dentifrice over the experiment and they were guided to suspend the use of fluoridation measurements, such as mouthrinses. After the *lead-in* period, the volunteers wore intraoral palatal device containing 4 restored root dentine slabs. On the first day, no erosive procedures were carried out to allow salivary pellicle formation. Starting on the second day, half of the palatal acrylic devices, with 2 root dentine slabs were immersed extraorally in a lemonade-like carbonated soft drink for 90 s, four times daily, for 3 days. During period of intraoral procedures, the volunteers had brushed only the inner part of their appliances with the same toothbrush and dentifrice employed to oral hygiene. At the end of the first intraoral phase, a 3-day wash-out period was founded, where the volunteers have not used the intraoral device. After the wash-out period, another 4 dentine slabs, restored with the alternative material, were placed into palatal appliance and the volunteers started the second phase of this study.

To avoid a possible carry-across effect,<sup>25</sup> in the first period, seven volunteers wore palatal appliance with dentine blocks restored with composite

resin and the other seven subjects inserted palatal devices loaded with dentine slabs restored with glass-ionomer cement. In the second period, volunteers were then crossed over to the alternative restorative material,<sup>26</sup> characterizing a 2x2 cross-over design (Figure 1).

#### Microhardness measurements around restorations

At the end of each intraoral phase, the specimens were positioned on acrylic rods with paralelometer and the measurements around the restorations were realized in a microhardness (HNV-2) testing with a Knoop diamond and a 25-gram static load which was applied for 10s. The surface microhardness measurements on root dentine were performed 300µm from the margin of the restored cavities.

#### Statistical methods

Results were analyzed by applying the ANOVA according to the split-plot model, after checking normality and homocedasticity. The differences among mean values were compared by using Tukey's test at significance level of 5%. The carry-over and period effects were evaluated by independent t test. The statistical analysis was performed with Statgraphics Centurion XV.

#### **Results**

The effects of carry-over were not statistically significant ( $p=0.255$ ) in contrast with period effects which were statistically significant ( $p=0.009$ ). Microhardness data were analyzed by split-plot ANOVA, which showed interaction between the main factors: dentine eroded / dentine uneroded and restorative material (glass-ionomer cement and composite resin) ( $p<0.0001$ ). The mean values and standard deviations for microhardness of the root dentine

eroded or uneroded restored with different restorative materials are summarized in Table I. Regardless of the restorative material employed, eroded specimens demonstrated lower microhardness values ( $p < 0.0001$ ). Within restorative materials, it was observed that, at any given dentine condition examined in this study, dentine restored with glass-ionomer cement showed higher microhardness values ( $p < 0.0001$ ).

### **Discussion**

Glass-ionomer cements are composed of fluoride-containing silicate glass and polyalkenoic acids which are set by an acid-based reaction between the components. During the setting reaction, a variety of ionic constituents is released from the glass, including fluoride.<sup>27</sup> Fluoride has been documented as a protector agent of the erosion progression in enamel and dentine<sup>20, 21, 28, 29</sup> however, the action of fluoride-containing restorative materials on development of root dentine erosion has not been evaluated yet. This study was delineated to evaluate the possible protective effect of fluoride from glass-ionomer cement in controlling the root dentine erosive lesions.

Microhardness measurements to evaluate differences between restorative materials were performed at 300 $\mu$ m from the margin of the restored cavities. The choice of the distance was based in previous studies that evaluate the extent of the cariostatic effect on root dentine provided by fluoride-containing materials<sup>30</sup> and the fluoride penetration from conventional glass-ionomer cements into dentine.<sup>31</sup> It was demonstrated that dentin microhardness values adjacent to Ketac fil were higher than the Z250 (control group) up to distance of 300 $\mu$ m for cariogenic challenges<sup>30</sup> and the incorporation of fluoride into dentine occurs at 300 $\mu$ m around restoration.<sup>31</sup>

The results of the current study showed that glass-ionomer cement was able to interfere with root dentine erosion progression, without completely preventing its development. The continual presence of a low concentration of fluoride appears to inhibit demineralization and enhance remineralization on eroded surface, as occurs for cariogenic challenges.<sup>30, 32, 33</sup>

Even though all volunteers have used fluoride-containing dentifrice during the experimental phase, the protective effect for root dentine erosion was shown only for specimens restored with glass ionomer cement. A possible explanation for this is the fact that the restored dentine was inserted in the mouth of the volunteers four days after the restorative procedures. Several studies revealed that the major fluoride release usually occurs within the first seven days.<sup>34, 35</sup> Thus, the fluoride effects might be amplified by the experimental conditions<sup>30</sup> since the erosive challenges were realized within this period. Thus, it is doubtful that glass-ionomer cements can have a significant erosive preventive effect over a longer period, because the amount of fluoride ions released from these materials decreases with the aging of the material.<sup>36</sup>

Previous studies evaluated the cariostatic effect of aging restorations and they demonstrated that freshly mixed glass ionomers significantly prevented enamel softening<sup>37</sup> however, the protective effect against demineralization disappeared with time.<sup>26, 37</sup> In other way, the disappearing fluoride effect could be reestablished by subsequent fluoride application.<sup>37</sup>

Daily exposition of filling materials to fluoridated dentifrices has demonstrated a high rechargibility for glass-ionomers, while the replenishment of resin based materials seems to be negligibly small.<sup>27</sup> Fluoride-releasing materials may act as a fluoride reservoir and may increase the fluoride level in

saliva, biofilm and dental hard tissues.<sup>27</sup> Probably, the glass ionomer cement would be capable to incorporate the fluoride from dentifrice and to maintain it for a long time in the oral cavity, favoring the remineralizing effect of the saliva and intervening which the acid challenges.

The saliva has been considered the most important biological factor influencing dental erosion due to its ability to act, directly on the erosive agent itself by diluting, clearing, neutralizing and buffering acids, play a role in forming a protective membrane, and to reduce the demineralization rate and enhance remineralization to eroded enamel and dentine.<sup>38</sup> However, saliva collected *in vitro* may undergo a number of changes which reduce its protective effect against acid degradation of tooth tissue, what highlights the important role of *in situ* models, particularly when dentine is tested.<sup>39</sup> Additionally, the temperature influences dentine erosion,<sup>40</sup> and all these factors were the main reasons for the use of an *in situ* study.

Similar study designs have been previously employed to evaluate the effect of fluoride on tooth erosion. For convenience, bovine dentine has been widely used in dental research as a substitute for human teeth.<sup>28</sup> Morphological differences exist between substrates, which results in higher rates of demineralization for bovine teeth, although the differences are only quantitative and not qualitative.<sup>41</sup> An enhancement in the lesion formation rate was also expected because polished surfaces were used, and they are more susceptible to acid challenges than natural surface.<sup>42</sup> However, polished surfaces are recommended to produce well-defined indentations in the surface microhardness test.<sup>43</sup>

The experimental protocol employed was based on previous intraoral investigations,<sup>23, 44, 45, 46, 47</sup> with slight modifications. The volunteers intakes were performed four times daily, since four or more nutritional acid intakes per day are associated with higher risk of development and progression of erosion.<sup>48, 49</sup>

Calcium, phosphate, pH value and to a lesser extent fluoride content of a drink are important factors explaining erosive attacks. Sprite Diet (pH 2.84 with CO<sub>2</sub>; citric acid, 18.4 g/100 ml; fluoride) was chosen as the demineralization solution because of its proven erosive capacity.<sup>46, 50</sup> Although samples had been extraorally demineralized, to avoid mineral loss from the volunteer's teeth, the detrimental influence of Sprite Diet was clearly demonstrated by the significantly lower SMH observed for the eroded groups.

Despite the statistical differences between periods, as shown in the t-test, the microhardness values adjacent to glass-ionomer cement were higher than that observed around composite resin restoration. Thus, the difference in periods may be ascribed to the volunteers hygiene habits and – or volunteers motivation,<sup>24</sup> but it did not affect the results of the current study.

Although both clinical and experimental observations show that individual wear mechanisms rarely act alone but interact with each other,<sup>51</sup> the abrasion by toothbrush was not performed on dentine restored slabs, since the response variable could be harmed and the possible effect of fluoride-containing restorative materials could be masked, as occurs in a previous study.<sup>52</sup> Future researches, with another variable response, could highlight the combined effect of erosion and abrasion on root dentine erosion formed *in situ*.

It is important to note that, *in vivo*, the pattern of the effect of fluoride-containing restorative materials on root dentine erosion is the result of a complex reaction among different chemicals, being dependent on many factors that could not be replicated in a *in situ/ex-vivo study*. Thus, supplementary research is required to investigate the influence of fluoride uptake of restorative materials, as well as the effect of the aging process of restorative material on root dentine erosion, close to clinical conditions.

### **Acknowledgments**

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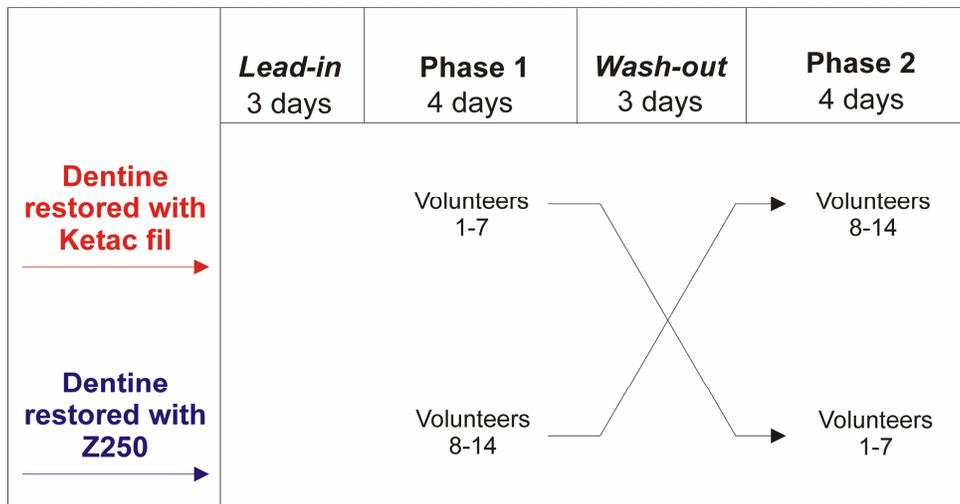
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Table 1. Means of microhardness values (KHN) and standard deviations of eroded and uneroded root dentine according to restorative material employed

|                  | <b>Uneroded</b> | <b>Eroded</b> |
|------------------|-----------------|---------------|
| <b>Ketac fil</b> | 52,7 (3.9) Aa   | 50.5 (7.6) Ab |
| <b>Z250</b>      | 53,0 (4.7) Aa   | 40.7 (4.3) Bb |

Capital letters - comparison within columns  
 Lower case - comparison within lines  
 Same letter indicates statistical similarity



**Figure 1.** Experimental design  
 (Adapted from Hara et al., 2006)

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<sup>N</sup> De acordo com as normas da Associação Brasileira de Normas Técnicas (ABNT) – NBR 6023.

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**ANEXOS**

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**ANEXOS**

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Prezado(a) Pesquisador(a),

**Ref.: Processo n. 2006.1.1048.58.6**  
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De ordem da Senhora Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em 72ª Sessão, realizada no dia 25 de outubro de 2006, deliberou **aprovar** o Projeto de Pesquisa envolvendo seres humanos intitulado: **"Microdureza da dentina radicular adjacente a materiais restauradores contendo flúor após desafios erosivos: Estudo *in situ*"**, a ser desenvolvido por Vossa Senhoria na Faculdade de Odontologia de Ribeirão Preto, devendo o atestado para publicação final ser expedido pelo Comitê de Ética em Pesquisa, após a entrega e aprovação do Relatório Final pelo referido Comitê.

Na oportunidade, lembramos da necessidade de entregar na Secretaria do Comitê, o **Relatório Final** até o dia **30 de abril de 2007**, com o formulário preenchido pelo pesquisador responsável conforme modelo que se encontra no *site* da FORP/USP (*link*: Comissões - Comitê de Ética em Pesquisa – Formulários do Pesquisador para entrega dos Relatórios Parcial ou Final).

Atenciosamente,

  
**Glaucia Della Rosa**  
Secretária do Comitê de Ética em Pesquisa

Ilma. Sra.

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