Faculdade de Odontologia de Piracicaba
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IDENTIFICAÇÃO MOLECULAR DA DENTINA DECÍDUA EM DIFERENTES TRATAMENTOS QUÍMICOS. ANÁLISE QUÍMICA, MICRO-MECÂNICA E MORFOLÓGICA.

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, como parte dos requisitos para obtenção do título de Doutor em Materiais Dentários.

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

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PROF. DR. MARIO ALEXANDRE COELHO SINHORETI

DEDICO ESTE TRABALHO

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"Quem tem por que viver agüenta qualquer como"

Nietzsche

RESUMO

Teoricamente, o suposto menor grau de mineralização da dentina decídua comparado à permanente, produziria comportamento diferente deste substrato após contato com soluções desproteinizantes/desmineralizantes, seguido de restaurações com materiais resinosos. O objetivo deste estudo foi fornecer novas informações científicas nos campos químicos, mecânico e morfológico em relação à qualidade do conteúdo mineral e orgânico da dentina decídua em comparação ao conteúdo da dentina permanente, após a ação de soluções desmineralizante/desproteinizante. No intuito de facilitar a apresentação desta tese, a mesma foi dividida em dois estudos, como descrito nas proposições seguintes. **Estudo 1:** Identificar a dentina decídua em comparação à permanente, na região de dentina média, após condicionamento com ácido fosfórico 35%. As análises foram: Espectroscopia Raman Transformada de Fourier (FT-Raman), Microscopia Eletrônica de Varredura/Espectroscopia de Energia Dispersiva (MEV/EED) e microdureza Knoop. Dez molares humanos, 5 decíduos e 5 permanentes, foram desgastados com fresa carbide até a dentina média e seccionados no sentido mesio-distal e vestíbulo-lingual, longitudinalmente. Metade da amostra foi destinada à espectroscopia FT-Raman e metade ao teste de dureza Knoop. Os grupos estudados foram (n=10): G1 (smear layer decídua); G2 (dentina decídua + ácido fosfórico 35%); G3 (smear layer permanente); G4 (dentina permanente + ácido fosfórico 35%). Espectros resultantes da análise química foram submetidos à análise de Cluster, com base nos componentes principais identificados. Análise MEV/EED foi realizada para complementar os dados obtidos pelo FT-Raman. O ensaio micromecânico de dureza foi realizado para cada grupo (n=5). Cinco impressões foram feitas em cada espécime com 100 µm de distância entre cada impressão. A média de cada grupo foi calculada e os valores foram submetidos à ANOVA e teste de Tukey ($p<0,05$). A análise FT-Raman revelou diferença entre os grupos. Conteúdo inorgânico*:* G3 foi o mais diferente, seguido pelo G4, enquanto G1 e G2 foram os mais similares entre si. MEV/EDS identificou os elementos químicos C, O, P, Ca e Zn. Conteúdo orgânico: G2 mostrou ser o mais diferente de todos os grupos, seguido pelo G4, enquanto G1 e G3 foram os mais similares entre si. O teste de dureza revelou não haver diferença significativa entre G1 vs G3 e G2 vs G4 ($p>0,05$), porém houve diferença significativa entre G1 vs G2 e G3 vs G4 ($p<0,05$). **Estudo 2:** Avaliar por meio da FT-Raman e MEV, a dentina coronária das

paredes laterais da câmara pulpar, decídua e permanente, após ação da solução NaOCl 1% seguido de condicionamento com ácido fosfórico a 35%, simulando as alterações que podem ocorrer em dentes tratados endodonticamente, os quais apresentam cavidade tipo classe I restaurada com compósito cujo sistema de união requer condicionamento ácido total. Vinte molares humanos, 10 decíduos e 10 permanentes, foram seccionados no sentido mesio-distal, longitudinalmente. As secções dentinárias foram distribuídas entre os grupos (n = 5): Dentes decíduos (Grupos 1, 2, 3 e 4); dentes permanentes (grupos 5, 6, 7 e 8) : G1 e G5 – dentina; G2 e G6 – dentina irrigada com NaOCl 1% (30 min); G3 e G7 - dentina irrigada com NaOCl 1% (30 min) e condicionada com ácido fosfórico 35%; G4 e G8 - dentina condicionada com ácido fosfórico 35%. Após a análise FT-Raman, todos os espécimes foram preparados e avaliados em MEV. As alterações morfológicas foram classificadas de acordo com score pré-estabelecido. **Resultados:** Conteúdo inorgânico: Houve nítida diferença entre a dentina que compõe a câmara pulpar decídua e permanente. G1 e G4 mostraram ser muito similares entre si, seguido do G2 que foi intermediário entre eles e G3. G6 e G7 também foram altamente similares entre si, seguido do G8 que foi intermediário entre eles e G5. Conteúdo orgânico: G7 e G8 foram muito similares entre si; G2 foi intermediário; G4 e G6 foram altamente similares entre si, bem como G3 e G5, os quais foram similares ao G1. As fotomicrografias demonstraram haver diferença morfológica entre os grupos não tratados, tratados com NaOCl e tratados com NaOCl seguido de condicionamento ácido, sendo que os grupos apenas condicionados não diferiram daqueles tratados e condicionados. **Conclusão Geral:** O arranjo molecular do conteúdo inorgânico dos dentes decíduos é qualitativamente diferente do arranjo molecular do conteúdo inorgânico dos dentes permanentes, tanto na dentina da região média coronária quanto da câmara pulpar, sendo o NaOCl um agente capaz de alterar o conteúdo inorgânico quimicamente, tanto na dentina decídua quanto na permanente, porém não morfologicamente caso seja tratado com NaOCl e seguido pelo condicionamento com ácido fosfórico.

ABSTRACT

Theoretically, it is supposed to have lower degree of mineralization for primary dentin compared with permanent one could lead different behavior of this substrate after contact with deproteinizating/demineralizating solutions, followed by composite restorations. The aim of this study was provide scientific informations at chemical, mechanical and morphological levels regarding the quality of inorganic and organic content of primary dentin compared to permanent one, as well as after deproteinizating/demineralizating solutions action. In order to facilitate the accomplishment of this thesis, it was divided into two phases, as described on the following descriptions: **Study 1:** To recognize the primary dentin compared to permanent dentin in the middle of crown, with or without 35% phosphoric acid total etching. The analyses were: Fourier Transformed Raman Spectroscopy (FT-Raman); Scanning Electron Microscopy/Energy-Dispersive Spectroscopy (EDS/SEM) and Hardness Micromechanical Test, related to molecular and micro-mechanical areas. Ten human molars, 5 primary and 5 permanent, were abrading with carbide bur until reach the middle dentin simultaneously to smear layer production. Four parts per tooth were obtained, half designed to FT-Raman spectroscopy and half to hardness test. The studied groups were (n=10): G1 (primary dentin smear layer); G2 (35% phosphoric acid etched primary dentin); G3 (permanent dentin smear layer); G4 (35% phosphoric acid etched permanent dentin). Result spectros from chemical analysis were subject to Cluster analysis based on identified principal components. The EDS/SEM analysis was done in order to add the FT-Raman data. To hardness test, 5 specimens were included, polished, cleaned, and etching (G2, G4) before the test. Five indentations were recorded to each specimen under middle dentin surface with 100 µm of distance each others beginning with 50 µm apart from JAD. A mean was calculated by each group and these values were recorded. The results were subjected to ANOVA and Tukey test. FT-Raman analysis showed difference among groups. Inorganic content: G3 was the most different, followed by G4, while G1 and G2 were the most similar to each other. EDS/SEM identified the C, O, P, Ca and Zn chemical elements in dentin. Organic content: G2 was the most different of all groups, followed by G4, while G1 and G3 were the most similar. There were no difference of hardness between G1 vs G3 and G2 vs G4 ($p>0.05$), however, there were difference between G1 vs G2 and G3 vs G4 ($p<0.05$). **Study 2:** FT-

Raman and SEM were used to evaluate chemically and morphologically the coronary dentin of pulp chamber laterals walls after 1% NaOCl action followed by 35% phosphoric acid total etching. The aim of this study was to simulate the changes that can occur in endodontically treated teeth with type class I cavity restored with composite and total etching adhesive systems. Twenty human molars, 10 primary and 10 permanent, were sectioned mesio-distally paralleled to long axis of teeth. Two parts of dentin pulp chamber, buccal and lingual lateral walls, were obtained from each tooth, providing 20 parts to primary as well as to permanent teeth, followed by division into groups (n = 5): Primary teeth (Groups 1, 2, 3 and 4); permanent teeth (groups 5, 6, 7 and 8) : G1 and G5 – pulp chamber dentin ; G2 and G6 – pulp chamber dentin irrigated with 1% NaOCl (30 min); G3 e G7 - pulp chamber dentin irrigated with 1% NaOCl (30 min) and etched by 35% phosphoric acid; G4 e G8 - pulp chamber dentin etched by 35% phosphoric acid. After FT-Raman analysis, all specimens were prepared and evaluated by SEM. The morphological changes were classified according a score. Results: Inorganic content: There was a clear difference between primary and permanent dentin pulp chamber. Within this division, G1 and G4 showed very similar profile between them, followed by G2 that was intermediated between them and G3. G6 and G7 showed are very similar between them, followed by G8 that was intermediated between them and G5. Organic content: G7 and G8 were very similar between them; G2 showed be intermediate; G4 and G6 were very similar between them as well as G3 and G5 that, in turn, were quite similar to G1. The photomicrographs showed difference among non-treated groups, NaOCl treated groups and NaOCl treated followed by phosphoric acid etched groups. The groups only acid etched not differ from NaOCl treated followed by acid etched groups. Therefore, the inorganic content of primary teeth is qualitatively different from inorganic content of permanent ones, both in middle dentin of crown as well as in pulp chamber dentin, but not morphologically in case of NaOCl treatment followed by phosphoric acid treatment.

SUMÁRIO

1. INTRODUÇÃO GERAL

A adequada união entre materiais restauradores resinosos e o substrato dentinário tem sido foco de diversas pesquisas que avaliam por meio de ensaios mecânicos se os valores de resistência de união são adequados ou não para serem posteriormente utilizados clinicamente (Inoue et al., 2001; Carrilho et al., 2004; De Munck et al., 2005; Hiraishi N et al., 2005). Porém, tão relevante quanto se avaliar mecanicamente a união substrato dentário/sistemas de união é o conhecimento detalhado do substrato sobre o qual esses materiais quimicamente balanceados são aplicados.

A dentina decídua apresenta menor grau de mineralização comparada ao dente permanente por apresentar o ciclo biológico reduzido (Araújo et al., 1995). Em média, a formação e mineralização da coroa de um dente decíduo são de no mínimo seis meses (incisivo central) e no máximo quatorze meses (segundo molar decíduo), enquanto a média de um dente permanente é de 3 a 4 anos. Além disso, com o início da rizólise, ocorre diminuição na formação da dentina reparadora ou terciária pelo órgão pulpar e os túbulos dentinários dos dentes decíduos não sofrem obliteração por deposição gradual de sais minerais na dentina peritubular (dentina esclerosada) (Johnson et al., 1969; Araujo et al., 1982;), como ocorre nos dentes permanentes. Frente a estas considerações conclui-se que o padrão de mineralização (velocidade e quantidade) do dente decíduo é cerca de 1/5 menor comparado ao dente permanente (Araújo et al., 1995).

Porém, estes relevantes estudos são histológicos e morfológicos, sendo que o conteúdo mineral da dentina decídua não foi quimicamente qualificado e quantificado em comparação ao dente permanente. Angker et al., (2003) & Angker et al. (2004) caracterizaram propriedades mecânicas da dentina decídua e seu conteúdo mineral, porém, sem a realização do grupo constituído por dentes permanentes. A comparação da dentina decídua e permanente em um mesmo trabalho é essencial, seja pela padronização do método de análise da pesquisa ou pela eliminação do fator de variação representado pelo operador. Uma vez que haja essa diferença estrutural entre ambas as dentições, a dentina decídua por apresentar menor conteúdo mineral e maior conteúdo orgânico, poderia se comportar diferentemente da dentina permanente frente ao uso dos sistemas de união contemporâneos.

Sabendo-se que a fase mineral deve ser removida do substrato dentinário sem causar danos na matriz de colágeno e os espaços deixados pelo mineral devem ser preenchidos com adesivo que se polimeriza *in-situ* e forma a camada híbrida (Nakabayashi et al., 1982), é de suma importância a caracterização da composição do substrato dentário no processo de união. A camada híbrida "ideal" deveria ser caracterizada como um biopolímero tri-dimensional de resina-colágeno que proporcionasse contínua e estável união entre o corpo do adesivo e o substrato dentinário (Misra et al., 2004).

Por maior que seja a interação física com a rede de colágeno exposta e química com o conteúdo mineral remanescente de alguns sistemas de união, a união dentinária com os monômeros resinosos hidrófilos e hidrófobos fatalmente se degrada. Essa degradação ocorre em função da umidade intrínseca do ambiente bucal, do substrato dentário (Pioch et al., 2001; Carrilho et al., 2004) e da água componente de sistemas adesivos auto-condicionantes (Tay et al., 2002; Reis et al., 2004), fenômeno conhecido como nanoinfiltração.

No entanto, a instrumentação da dentina por desgaste e outros métodos de preparo cavitário, alteram a superfície e formam a *smear layer*, a qual cobre os componentes estruturais normais da dentina. A *smear layer* da dentina coronária é composta basicamente por debris dentinários (Taylor et al., 1997) e difere da dentina radicular com relação a quantidade de debris, mais severamente formados na última, dada à instrumentação inerente ao tratamento endodôntico (Ozturk et al., 2004).

A análise da *smear layer* tem sido explorada na literatura (Pashley et al., 1988; Burrow et al., 1996), uma vez que seu papel é um fator que contribui para a formação da camada híbrida e cuja característica tem grande peso na busca da camada híbrida "ideal" ou menos degradável. Na grande maioria os trabalhos são de caracterização morfológica, os quais constatam que o ácido fosfórico utilizado para condicionar as superfícies dentinárias remove a *smear layer*. Segundo Spencer & Wang, (2001), até então, trabalhos que evidenciassem as alterações químicas causadas na *smear layer* pela ação do ácido fosfórico não tinham sido realizados para dar suporte às pesquisas de análise morfológica, as quais dão luz à descrição das estruturas observadas, mas não disponibilizam dados em níveis moleculares.

Restaurações utilizando-se compósito em dentes tratados endodonticamente têm sido amplamente empregadas. Segundo Ausiello et al. (1997), dentes permanentes tratados endodonticamente e restaurados adesivamente apresentaram resultados similares aos dos dentes hígidos quando avaliados quanto à resistência à fratura. No entanto, em se tratando da dentina da câmara pulpar decídua e permanente, não há trabalhos na literatura que especifiquem o quão diferente são estes substratos no aspecto químico e mecânico.

Em relação à resistência de união de monômeros resinosos nas paredes remanescentes da câmara pulpar, Akagawa et al. (2002) & Toba et al. (2003), demonstraram que o adesivo Single Bond apresentou camada híbrida mais espessa no assoalho da câmara pulpar em comparação à dentina coronária e o adesivo Clearfil SE Bond produziu espessuras similares da zona de hibridização nas duas regiões da dentina. Vale ressaltar que o primeiro material requer condicionamento ácido total e o segundo material é composto de primer autocondicionante.

A solução de hipoclorito de sódio (NaOCl) promove a remoção de colágeno (Ciucchi et al., 1989) e alterações produzidas na dentina podem afetar a qualidade da união adesiva às paredes laterais e ao assoalho da câmara pulpar. Segundo Morris et al. (2001), é provável que o NaOCl oxide algum componente da matriz dentinária, o que dificultaria o início da polimerização de sistemas resinosos, proporcionando a diminuição da resistência de união (Nikaido et al., 1999; Morris et al., 2001; Ari et al., 2003; Ozturk et al., 2004). Em análise morfológica da dentina permanente mineralizada, a aplicação de NaOCl a 5% causou a exposição de poros na superfície e numerosos canais que não seriam normalmente visualizados (Marshall et al., 2001; Puppin-Rontani & Caldo-Teixeira, 2003), remoção de fibrilas de colágeno, deixando a superfície da dentina lisa e ausência da zona de hibridização em interfaces dentina/resina (Ozturk et al., 2004). Sim et al. 2001, observaram a diminuição de propriedades mecânicas da dentina permanente como módulo de elasticidade e resistência flexural frente à ação do NaOCl 5,25%. Este agente considerado apenas desproteinizante reduziu o conteúdo de matriz orgânica quando usado na concentração de 5% (Moutouris et al., 2004) e erradicou os modos amina e água na concentração de 13%, além de alterar o conteúdo mineral (Tsuda et et al., 1996), em dentes permanentes.

Borges et al. (2005), avaliaram a recuperação da resistência à compressão em dentes decíduos restaurados com *onlays* de compósito após simulação da irrigação com NaOCl a 1%. Os autores observaram que não houve diferença entre os dentes restaurados e hígidos, independentemente do tratamento com NaOCl a 1%. No entanto, este tratamento apresentou resultados significativamente diferentes em relação à resistência de união à microtração e selamento pulpar em dentes permanentes (Ozturk et al., 2004, Ozturk et al., 2004 b). Faz-se necessário a exploração de métodos diretos de análise química das alterações do substrato dentinário decíduo. Assim sendo, esta tese propõe avaliar qualitativamente o conteúdo mineral e orgânico da dentina decídua em comparação ao conteúdo da dentina permanente, no aspecto químico, mecânico e morfológico, bem como após a ação de soluções desproteinizantes/desmineralizantes.

2. PROPOSIÇÃO

Esta tese foi dividida em dois artigos que estão contemplados nos capítulos 1 e 2.

Os objetivos deste estudo foram:

1. Identificar o substrato dentinário decíduo em comparação com o permanente, na região de dentina média, com e sem condicionamento com ácido fosfórico, nos campos molecular e micro-mecânico (Capítulo 1);

2. Identificar química e morfologicamente a dentina da câmara pulpar do substrato decíduo comparando-o com o permanente, frente ao tratamento com NaOCl a 1%, seguido condicionamento com ácido fosfórico (Capítulo 2);

Este trabalho foi realizado no formato alternativo, com base na deliberação da Comissão Central de Pós-Graduação (CCPG) da Universidade Estadual de Campinas (UNICAMP) n°. 001/98.

3. CAPÍTULOS

3.1 CAPÍTULO 1

"Novas perspectivas sobre o arranjo molecular da dentina decídua e permanente"

"New perspectives about molecular arrangement of primary and permanent dentin"

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Abstract

The dentin quality of primary and permanent teeth was inspected by Fourier Transformed Raman Spectroscopy (FT-Raman); Scanning Electron Microscopy/Energy-Dispersive Spectroscopy (SEM/EDS) and Hardness Test. Middle dentin of crowns were reached by carbide bur abrading providing a uniform smear layer. Phosphoric acid was applied in order to simulate the etching of total etching adhesive systems. The groups were (n=10): G1 (primary dentin smear layer); G2 (35% phosphoric acid etched primary dentin); G3 (permanent dentin smear layer); G4 (35% phosphoric acid etched permanent dentin). FT-Raman results were subjected to Cluster Analysis. SEM/EDS were made in order to add the data obtained by FT-Raman. The hardness data were subjected to ANOVA and Tukey test. FT-Raman showed differences among groups, either to organic or inorganic content. For the organic content, primary and permanent dentin became similar after the etching; conversely, the inorganic content showed differences for the two substrates. Hardness test showed no significant differences between primary and permanent dentin, before or after etching, but the etching decreased these values. The mineral content arrangement of primary dentin is different from permanent dentin, independently of the etching. The substrate type did no influence the hardness, but the etching decreased it.

PACS: 47.11.Kb; 47.11.Mn

Keywords – dentin, mineral content, FT-Raman, hardness.

INTRODUCTION

Primary teeth present structural differences from permanent teeth, such as lesser thickness of dentin and proportionally bigger pulp chamber than its successor teeth. The primary crown average growth is from six to fourteen months, whereas the permanent teeth average growth is from three to four years [1]. Additionally, the dentin secretion and pulpar repair activity of primary teeth decreases with aging [2].

The rate of in vitro caries progression was significantly higher in primary enamel when compared to permanent enamel [3,4]. Concerning dentin caries progression rates, it was observed in an in vivo study that for 9-year-old children, the rate of caries progression was on average 3.6 times higher for second primary molars than for first permanent molars [5]. Concerning mechanical properties, hardness, plastic hardness and modulus of elasticity from canines' primary dentin, their values were significantly lower than premolars dentin at most sites, decreasing from outer toward the inner layers [6].

The dentin mineralization quantity has a role to the bond strength of composite restorations to dentin. The mineral phase should be dissolved causing no damage in the exposed collagen matrix, which is infiltrated by resin monomers which polymerize [7]. The ideal hybrid layer would be characterized as a three-dimensional collagen-resin biopolymer which provides a continuous and stable link between the bulk of the adhesive and the dentin substrate [8]. Efforts have been done to identify those prejudicial factors on the formation of an ideal hybrid layer mainly in permanent dentin [9-11]. Concerning primary dentin, first it is necessary to clarify the compositional features and whether it differs truly from the permanent dentin.

What is really different in dentin composition between primary and permanent teeth? The role of dentin organic matrix should not be disregard in caries and hybridization process with adhesive systems. Would the mineral content or the organic matrix be the mainly responsible for different behaviors of primary and permanent dentin? Studies qualifying the mineral and organic matrix differences between primary and permanent dentin are absent in the worldwide literature.

A Raman spectroscopy study in permanent teeth, suggested that the collagen within the acid-treated smear layers is disorganized and denatured, which forms a gelatinous matrix around portions of mineral trapping them and shielding them from the

acid [12]. In a structure not well recognized as primary dentin, the effect of phosphoric acid could be different from permanent dentin.

This study investigated the effects of 35 % phosphoric acid etching on primary and permanent dentin. It was hypothesized that (1) chemical composition arrangement (organic and inorganic contents) are different for both primary and permanent teeth; (2) mechanical behavior is different for primary and permanent dentin before and after acid etching; (3) the arrangement of chemical composition is changed by acid etching either in organic or inorganic contents to primary and permanent dentin.

MATERIALS AND METHODS

Selection of teeth

Sound human third molars and primary molars frozen stored within 1 month of extraction were used in this study. The teeth were collected after patients´ informed consent was obtained under a protocol approved by the Research Ethics Committee of FOP/UNICAMP (Approval No. 083/2005) according to the Resolution of the National Commission of Ethics in Research. Five mandibular second primary molars and five third molars with no caries, composite restorations or any abnormality were selected after inspection and confirmed by digital radiographies (Cygnus X- Ray; Cygnus, San Francisco, CA, USA). The roots of third molars were sectioned and discarded. The primary teeth did not present roots due to the exfoliation process.

Determination of dentin depth

Previously to the digital radiographies, a small fragment of wire steel was fixed with wax at a proximal surface of each tooth in order to be used as reference to measure the dentin depth which each crown would be reach, as follows the explanation. The wire was measured using a digital caliper rule (Mitutoyo, Tokyo, Japan) and its measure was recorded to be used in the Image Tool 3.0 software. The Image Tool 3.0 software (Periodontology Department, University of Texas, and Health Science Center at San Antonio, TX, USA) was used to determine the dentin depth to be reached by carbide bur abrading. The command *Calibrate Spatial Measurements* of Image Tool Software was assessed and the measure of the wire steel upon the proximal tooth surface was required into a specific box. After to write the wire steel measure into the box and press *ok*, every

measurement done upon the digital radiography was transformed to millimeters. This procedure was repeated to each radiography.

The *Distance* command of Image Tool Software was put in action and a line was drawn upon the tooth from the top of a higher cusp until the middle third of the crown, based on the lesser radiopacity of dentin, which differentiated it from enamel and from pulp chamber that is more translucent. All measurements were recorded.

Specimen preparation

Each tooth was fixed with wax by the cervical surface upon acrylic plates. The middle dentin depth was determined by transference of recorded values previously measured by digital radiographies. One black line was drawn upon the buccal surface of each crown. The occlusal surface was abraded with 57L carbide bur (KG Sorensen, São Paulo, S.P., Brazil) until it reached the black line on the buccal surface in a high-speed handpiece under copious air-water spray. The handpiece was positioned in a machine in order to standardize the flat dentin area and the smear layer. Each tooth was bucco-lingually and mesio-distally sectioned by the use of a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL, USA). They were sectioned paralleled to the long axis of tooth. Four parts per tooth were obtained. Each part was individually sonicatted (Maxiclean 750, Unique Ind. e Comp., Indaiatuba, SP, Brazil), immersed in deionized water for 5 minutes in order to remove any residual impurities. Half of the sample was separated to molecular analysis and half was selected to mechanical test. The parts were distributed to experimental groups (n=5) according to substrate (primary and permanent) and treatment (with or without acid etching), allowing the identification of the source of each part, as follows: G1 – primary dentin + smear layer; G2 – primary dentin + 35% phosphoric acid treatment*; G3 – permanent dentin + smear layer; G4 – permanent dentin + 35% phosphoric acid*.

The 35% phosphoric acid (pH 0.6) was manipulated in liquid form by Biochemistry Laboratory of Piracicaba School of Dentistry, UNICAMP, São Paulo, Brazil*, in order to avoid the colloidal silica present in the phosphoric acid gel form to damage the spectroscopy measurements and micrographics images as shown by a pilot study (unpublished data). The specimens were etched for 15s and rinsed with tapered water

for 10s. Each specimen was stored immersed into Karnovisk solution in order to fix and preserve the exposing collagen of the dentin.

Fourier Transformed Raman Spectroscopy (FT-Raman) (n=5)

The molecular measurements were carried out on FT-Raman Spectrometer RFS 100 (Bruker Optics Inc, Billerica, MA, USA) equipped with OPUS software (4.2 version, Copyright © Bruker Optics GmHb 1997-2002, Billerica, MA, USA) at University Vale do Paraíba (UNIVAP, São José dos Campos, SP, Brazil).

The spectra were recorded at a 4 cm^{-1} spectral resolution of; 7 mm laser beam aperture by the use of a focused 1064 ηm line. The Nd:YAG near-IR laser was used as an excitation line. The maximum laser power incident on the specimens was ~ 360 mW. The specimens were examined upon sampling plate fixed by double face adhesive tape, perpendicular to the laser beam. One measurement was carried out upon the middle flat dentin with 1 mm^2 scanning area and ~ 6 min integration time, at room temperature.

Scanning Electron Microscopy/ Energy-Dispersive Spectroscopy (SEM /EDS)

All sample analyzed by FT-Raman was prepared according to Perdigão et al., 1995 protocol of dentin sample fixation and dehydration. Carbon evaporation was performed on a Denton Vacuum, Desk II (Buffallo, NJ, USA). Micrographs (x1000 magnification) were obtained by Scanning Electron Microscopic JEOL JSM-5600LV (Tokyo, Japan) operating at 15 kV, 15 mm work distance and spotsize 38. These samples provided the spectral analysis by EDS Vantage device (version 1.4 Rev. B, Noran Instruments, Tokyo, Japan). Three areas of the same specimen were evaluated and the chemical elements were automatically identified in order to add the data obtained by FT-Raman.

Hardness Test (n=5)

The parts of the teeth selected to the hardness test had its middle dentin surface fixed to the red wax, in order to join 5 specimens to each group (G1, G2, G3, G4) and to put them together into PVC cylinders (27 mm diameter). Next, polystyrene resin (Piraglass Ltda., Piracicaba, S.P., Brazil) was poured into the cylinders. After the polymerization, the surface of each set was flattened by silicon carbide sandpaper (#600) in a politriz device (APL-4, Arotec, São Paulo, S.P., Brazil) followed by a 10 min sonication (Maxi Clean 750 - UNIQUE UltraSonic, Indaiatuba, S.P., Brazil). The groups 2 and 4 sets were etched with

phosphoric acid for 15 s, rinsed with tapered water for 10 s, similarly to the specimens of the FT-Raman and the SEM/EDS methods.

The hardness machine HMV2 (Shimadzu, Tokyo, Japan) containing a Knoop diamond was used. A load of 25g was applied for 15s. Five indentations were recorded to each specimen on the middle dentin surface. The distance between the first indentation was 50 µm from amelodentin junction and 100 µm from another one. A mean was calculated by each group and these values were recorded.

Statistical Analysis

To verify intra-specimen variation, FT-Raman spectrum was recorded in three different regions in two specimens of each group. It was observed that the spectral patterns were exactly the same and there were no difference after Cluster Analysis application (see explanation below) and thereafter it was established that one measurement for each specimen was legitimate. The entire spectrum in the range ~ 400 to 3000 cm^{-1} was selected since it included the main bands related to both organic and inorganic structures of dentin.

In this study, were analyzed twenty Raman spectra of human dentin divided into four groups: G1, G2, G3, and G4. Cluster analysis was performed using data pretreatment techniques, that is a combination of smaller regions of the organic and inorganic regions of the dentin Raman spectra, which were most discriminative to achieve optimal classifications. The bands are a set of wavenumbers which contains a main wavenumber related to the peak of the band. The wavenumbers of the peaks were related to the molecules of dentin and confirmed by international literature [12, 14-15]. The peaks used in this study were related to the Principle Components Identified (PCIs). The peaks of the organic content were: 1254, 1456, 1667, 2942 cm^{-1} related to PCIs C-H bonds, amide I, CH^2 bonds, amide III, and for the inorganic content the following peaks: 431, 590, 856, 875, 960, 1074 cm^{-1} related to PCIs CO_3^{2-}, $PO_4^{3-,}$ P-OH, PO_4^{3-} bonds. The spectra of each group were composed by 727 data points in the spectral range 400 to 1800 cm^{-1} and were divided into organic and inorganic spectra. The organic bands, assigned in this study, were chosen at 1234-1274, 1434-1477, 1646-1689, 2921-2963 cm^{-1}, and the inorganic bands at 410-453, 568-611, 834-896, 938-981, 1050-1093 cm^{-1}, providing approximately 22 data points to each assigned band. Principle Components Analysis was used as patterns detection technique to reduce the organic and inorganic bands data for improvement of the

discrimination capacity of the software (OPUS software (4.2 version, Copyright © Bruker Optics GmHb 1997-2002, Billerica, MA, USA). Clustering techniques of the individual spectrum were applied to achieve groups according to spectral similarity. Cluster analysis was performed using the all principal components identified. To organic content, the spectrum collect was since the wavenumber of the C-H band beginning until the wavenumber of amida III band end. To inorganic content, the spectrum collect was since the wavenumber of the CO_3^{2-}, PO_4^{3-} band beginning until the wavenumber of the other PO_4^{3-} band end. All spectra ranges used were equally weighted, and Average Linkage algorithm was applied. For calculating the distance matrix, Factorization was used. For a better visualization of all analysis, dendrogram of hierarchical cluster analysis were performed.

ANOVA Two-Way and Tukey Tests were performed to evaluate the Hardness micromechanical test data.

RESULTS

FT-Raman

In this study, the mean spectra of the groups have the same baseline; however, they were dissociated in order to improve the visualization of each spectrum (fig 1a, fig 2a). The spectrum features were very similar among groups with no visible broadening of any band (fig 1a, fig 2a). However, the statistical analysis showed that there were differences among groups as can be observed by the dendrograms, for both organic and inorganic content. Dendrograms are formed by principal root that is dissociated into right and left sides. When groups are in the same side but present different spectral distances in the baseline among them, they are statistically similar. However, when groups are in the same side and present the same spectral distance in the baseline they are highly similar among them. In opposite, when groups are into different sides within keys with different spectral distance values, they are statistically different among them.

Figure 1b shows that G2 and G4 are at the right side of the principle root, which means that despite the G2 (with spectral distance near to 1.4), have a spectral distance difference ~ 0.4 to G4 (with spectral distance near to 0.6) they are statistically similar. On the other hand, G1 and G3 were highly similar (within a same key at the left side of the

principle root), showing the same spectral distance, that, in turn, they were statistically different from G2 and G4.

To mineral content, G4 and G3 are at the right side of the principle root, which means that despite the G4 (with spectral distance near to 0.4), have a spectral distance difference ~ 0.6 to G3 (with spectral distance near to 1) they are statistically similar. On the other hand, G1 and G2 were highly similar (within a same key at the left side of the principle root), showing the same spectral distance, that, in turn, they were statistically different from G2 and G4 (fig 2b).

EDS/SEM

The spectros obtained by EDS/SEM identified Ca, O, P, Z (fig 3a, 4a). The spectros obtained reveled that there is no difference between primary and permanent middle dentin concerning individuals chemical elements.

Hardness Test

There were no significantly differences between G1 versus G3; and G2 versus G4 ($p>0.05$). The lack of difference showed the similarity of hardness values of primary and permanent dentin. On the other hand, there were significant differences between hardness values of G1 vs G2; and G3 vs G4 ($p<0.05$), because of the phosphoric acid action (Table 1).

DISCUSSION

The Cluster Analysis, supported by Principal Components Analysis (PCA) among groups is a reliable method of evaluating the FT-Raman spectrum because it can show significant differences that are difficult to be seen only through spectral exam. In general, spectroscopic studies analyze the bands width. For example, if a peak is thin the molecule is completely formed; conversely, if a peak is broad changes can occur in the molecule structure [12, 14-15]. In this study, the spectra did not show visible differences among studied groups, as a broadening of the bands. The limitation of this study was the 20 μm laser penetration depth. This depth included both the smear layer (4 μm according Spencer et al., 2001) and the subjacent dentin available to adhesion. However, the Cluster Analysis showed that there were changes to both organic and inorganic content.

Noticeably, organic and inorganic contents were distinct within the wavenumber studied. They were separately analyzed, which was one of the objectives of this study. The organic peaks identified were 1,254 cm^{-1} (amida III), 1,456 cm^{-1} (CH_2), 1,667 cm^{-1} (amida I), and 2,942 cm^{-1} (CH). The inorganic peaks identified were 431 cm^{-1} (PO_4^{3-}), 590 cm^{-1} (PO_4^{3-}), 875 cm^{-1}, and 856 cm^{-1} corresponding to P-OH stretch, 960 cm^{-1} (PO_4^{3-}), and 1,074 cm^{-1} related to both PO_4^{3-} and CO_3^{2-} bonds [12, 14-15] (fig 1a, fig 2a).

The first hypothesis was partially proved: both primary and permanent dentin present similar arrangement of organic component (fig 1b). However, they showed difference in mineral content (fig 2b). This study showed that mineral content was the only responsible for the chemical differences between primary and permanent dentin. G3 (permanent dentin smear layer) showed the most significant difference from the other groups but it showed to be similar to G4 (etched permanent dentin); G1 (primary dentin smear layer) and G2 (etched primary dentin) showed to be highly similar to each other (fig 2a and 2b). The mineral content of permanent dentin was more sensible to phosphoric acid action compared to mineral content of primary dentin, which showed the same spectral distance.

These findings could be probably related to the age of the compared teeth. The aging process can modify the dentin mineral structures [16-18] because it is a natural process. It is based on chemical reactions. Although the third molar growth time is approximately three times higher than the primary second molar growth time [1], primary tooth is a substrate prepared to have a short period of function in the mouth and probably, its mineral content chemical reactions might be higher than the permanent tooth. The higher the mineral crystallinity of dentin, the lower carbonate to phosphate ratio [19]. The aging process decreased the apatite 960 cm^{-1} peak width indicating an increase in apatite crystallinity [20]. In this work, the identified 1074 cm^{-1} peak is related to type-B carbonate substitution, i.e., CO_3^{2-} substitution for PO_4^{3-} . The degree of crystallinity can increase in two different ways, either the atoms change their sites making the crystal more perfect or the individual mineral crystal becomes larger [21].

These findings of the literature contributed to explain the invalidation of the second hypothesis of this study. The mechanical behavior (hardness) was similar between primary and permanent dentin, before and after acid etching (Table 1). Whether primary

dentin is really lesser mineralized than permanent dentin, the increase of apatite crystallinity over time can compensate this difference providing similar mechanical strength to the old primary teeth. The increased crystallinity of primary dentin with aging becomes more resistant to plastic deformation [19]. Furthermore, the smear layer filled the tubule lumens and this can contribute to the increase of dentin hardness [18]. The tubules orientation also can influences the mechanical properties of dentin. There is a small transverse isotropic symmetry with the stiffest direction being perpendicular to the tubules [22] which is the same direction of middle dentin evaluated in this study.

In spite of the dentin secretion and pulpar repair activity decreasing with aging in primary teeth [2], the physiological wear from mastication provides the deposition of apatite crystal in lumen tubules over time. This crystalline deposition seems to be a process involving dissolution of intertubular mineral, followed by re-deposition into the tubules [16]. It may be speculated that the mastication might cause the reduction of the dentin mantle thickness and globular dentin in aged permanent teeth when compared to young permanent teeth, decreasing the fracture strength [16,17] and increasing the hardness and modulus of elasticity of the aged dentin [18].This chemical mechanism of crystalline deposition during aging is still uncertain, however, the Ca/P ratio of crystallites from the sclerotic casts within the dentinal tubules is slightly lower than the calculated value of 1.50 for tricalcium phosphate [23]. The additional presence of about 5% magnesium suggests that these crystallites are whitlockite (Mg substituted b-tricalcium phosphate) [24]. In this study, the spectra obtained by SEM/EDS did not show any element that could replace the Ca^{2+}, PO^{3-}_{4} or OH^{-}, neither in primary (fig 3a, fig 3b) nor in permanent specimens (fig 3c, fig 3d).

Nevertheless, the 35% phosphoric acid decreased the hardness of both primary and permanent dentin (table 1). Despite factors such as the buffering effect of hydroxyapatite (and other dentin components) and the acid hypertonicity that restricts the interaction of the etching agents with dentin [25-27], the acid reaction with dentin occurs and provides an etched dentin ready to adhesive procedures. The inorganic content of dentin is removed exposing the superficial organic matrix layer, the tubules lumens, and creating porosities which result in a dentin more resilient and less hard [28,29].

Moreover, the tubule density of third permanent molars in the middle dentin is 37,000 tubules/mm^2 [30] and for second primary molars (used in this study) also in the middle dentin, the tubule density is 25,300 tubules/mm^2 [31]. The lesser tubule density of second primary molar can be one factor that contributed to the hardness of primary and permanent dentin to be similar. This provides a higher intertubular dentin thickness. In addition, primary peritubular dentin is two to five times thicker than the permanent dentin [32].

The third hypothesis was partially validated: 35% phosphoric acid did not change the inorganic content analyzed by Cluster Analysis (fig 2), but modified the organic content quality for both primary and permanent dentin (fig 1). The inorganic content of the smear layer was removed from primary and permanent dentin by acid etching, but the inorganic content of the subjacent dentin layer showed difference between both substrates as discussed above. Concerning the dentin organic matrix (in fig 1b), it can be observed that G2 (etched primary dentin) was statistically the most different. On the other hand, G1 (primary dentin smear layer) and G3 (permanent dentin smear layer) had their organic content highly similar. The organic content of primary dentin performed similarly to the organic content of permanent dentin after phosphoric acid action.

Phosphoric acid is not a solvent for the organic portion of the smear layer [12], but the chemical changes were detected by Cluster Statistical Analysis after 35% phosphoric acid etching to both primary and permanent dentin. The dentin has a chemical structure that dictates its behavior under situations such as acid etching. Therefore, the acid etching of the dentin partially solubiblizes two types of proteins from the matrix. The first class includes acidic noncollagenous phosphoproteins and glycosaminoglycans (GAGs). The other class of proteins is neutral polymers such as low molecular weight peptides, including solubilized low molecular weight collagen [33]. The most important changes which can occur in the etched dentin are the decrease of the mineral bands and consequently the increase of the organic bands [12]. These features in this study were not visible detected by spectra analysis, but possible detected by Cluster Statistical Analysis. When compared the spectrum of carbide bur smear layer and completely demineralized dentin by phosphoric acid, the spectral features associated with amides I and III are broadened [12].

In this study, the collapse of the collagen matrix was minimized because of the absence of the bond procedure air drying step. Demineralization causes a 19% reduction in dentin volume versus a 65.6% after demineralization followed by air-drying [34]. However, during the spectroscopy analysis, the specimens, still out of water, become naturally dried; they can not be considered collapsed as when they are air dried, but changes of dentin matrix proteins could have begin earlier.

The chemical changes might have occurred for two reasons. Firstly, the components of Karnovisk solution (glutaraldehyde and phosphate-buffered saline), commonly used to preserve the organic structures in laboratory studies, can stiffen the acid etched demineralized dentin matrix and consequently increase the hardness and modulus of elasticity of specimens both measured by isometric contractile activity [35]. It was not determined whether the molecular mechanisms responsible for the isometric contraction represent a form of denaturation of collagen. The collagen denaturation is the loss of its morphological integrity [36]. This phenomenon might be related to chemical changes too, not simply a physical process, if any alteration occurred with triple helices of collagen by Karnovisk solution interaction.

Secondly, degradation of demineralized dentin collagen probably could have been sufficient to lead to chemical changes molecularly detectable. The matrix metalloproteinases (MMPs) that are within the mineralized dentin, containing collagenolytic and gelatinolytic activities (MMP-2 and MMP-20), can be released and activated by low pH [37-39]. The phosphoric acid gel at 37% for 15 s decreased its intrinsic mineralized dentin powder collagenolytic activity because of its low pH, which could denaturate the MMPs or inactivate it by calcium ions removal [40]. Despite this fact, the concern about the action of phosphoric acid at concentrations commonly used in adhesive bonding procedures has been still. There is dentin collagen hydrolytic degradation with no bacterial colonization over time in aqueous environment [40]. Moreover, the simplified etch-and-rinse adhesives can reactivate endogenous enzymatic activities in dentin previously inactivated by phosphoric acid-etching [41].

The current study reinforces the need of discovering a way to prevent the exposed collagen fibrils from acid etched dentin to become degradated to both primary and permanent dentin. There are evidences of MMPs actives in primary dentin because of there

was a decreasing of resin-dentin interface degradation in primary teeth when chlorhexidine was used after etching [42]. Since there were no differences between organic content of primary and permanent dentin, the collagen matrix of primary dentin seems as susceptible to degradation as collagen matrix of permanent dentin [40,41].

The behavior of primary and permanent dentin seems to be different under conditions such as caries process and bond strength. Dentin caries rates were 20.5 new lesions/100 tooth surface-years for mesial face of permanent first molars compared to 32.6 for primary second molars [5]. Concerning adhesive restorations, the overall bond strengths of different adhesive materials were greater for permanent dentin compared to primary dentin [43]. How would the chemical behavior of primary and permanent dentin be submitted to other chemical solutions used in Dentistry? Studies have been carried out to know the behavior of both primary and permanent dentin after contact with sodium hypochlorite used in pulp therapy and other etchings used to composite restorations, such as self-etching primers and one-bottle adhesive systems. It is needed to get more information about these issues followed by ways that minimize the intrinsic factors of primary dentin which damage its behavior in oral cavity.

CONCLUSIONS

Within the limitations of this study it can be concluded that:

1. The inorganic content molecular arrangement of primary dentin is different from permanent dentin.
2. The hardness was similar to primary and permanent dentin, decreasing to both only after acid etching.
3. The organic content is modified by acid etching in primary dentin as well as in permanent dentin.

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REFERENCES

[1] F.B. Araújo, F.F. Moraes, A.C.M. Fossati, Rev. Bras. Od. 52 (1995) 37.

[2] D.V. Johnsen, in: JK Avery (Ed), Oral development and histology. New York : Thiema Medical (1994) p.154.

[3] J.D. Featherstone, J.R. Mellberg, Caries Res. 15 (1981) 109.

[4] R.P. Shellis, Arch. Oral Biol. 29 (1984) 975.

[5] I. Mejàre, H. Stenlund. Caries Res. 34 (2000) 454.

[6] Y. Hosoya, G.W. Marshall, J. Mater. Sci. Mater. Med. 16 (2005) 1-8.

[7] N. Nakabayashi, K. Kojima, E. Masuhara, J. Biomed. Mater. Res. 16 (1982) 265.

[8] A. Misra, P. Spencer, O. Marangos, Y. Wang, J. Katz, J. Biomed. Mater. Res. B Appl. Biomater. 70 (2004) 56.

[9] M.R. Carrilho, R.M. Carvalho, F.R. Tay, D.H. Pashley, Am. J. Dent. 17 (2004) 104.

[10] N. Hiraishi, N. Nishiyama, K. Ikemura, J.Y. Yau, N.M. King, J. Tagami, D.H. Pashley, F.R. Tay, J. Dent. Res. 84 (2005) 653.

[11] Y. Wang, P. Spencer, J. Biomed. Mater. Res. 59 (2002) 46.

[12] P. Spencer, Y. Wang, M.P. Walker, J.R. Swafford, J. Dent. Res. 80 (2001) 1802.

[13] J. Perdigão, P. Lambrechts, B. Van Meerbeck, G. Vanherle, A.L. Lopes. J Biom. Mat. Res. 26 (1995) 1111.

[14] H. Tsuda, J. Arends, J. Dent. Res. 73 (1994) 1703.

[15] G. Penel, G. Leroy, C. Rey, E. Bres, Calcif. Tis. Int. 63 (1998) 475.

[16] R.K. Nalla, A.E. Portes, C. Darario, A.M. Mnor, V. Radmillovia, E.A. Stach, A.P. Tomsia, R.O. Ritchie, Micron. 36 (2005) 672.

[17] J.H. Kinney, R.K. Nalla, T.M. Pople, T.M. Breunig, R.O. Ritchie, Biomat. 26 (2005) 3363.

[18] P. Senawongse, M. Otsuki, J. Tagami, I. Mjor, Arch. Oral Biol. 51 (2006) 457.

[19] W. Tesh, N. Eidelman, P. Roschger, F. Goldenberg, K. Klaushofer, P. Fratzi, Calcif. Tissue Int. 69 (2001) 147.

[20] J.J. Freeman, B. Wopenka, M.J. Silva, J.D. Pateris, Calcif. Tissue Int. 68 (2001) 156.

[21] S. Weiner, W. Traub, Fed. Am. Soc. Expl. Bio. J. 6 (1992) 879.

[22] J.H. Kinney, R.K. Nalla, J.A. Pople, T.M. Breunig, R.O. Ritchie, Biomat. 26 (2005) 3363.

[23] K. Ishikawa, T. Suge, M. Yoshiyama, A. Kawasaki, K. Asoaka, S. Ebisu, J. Dent. Res. 73 (1994) 1197.

[24] F.R. Tay, D.H. Pashey, J. Dent. 32 (2004) 173.

[25] J.D. Wang, W.R. Hume, Int. End. J. 21 (1988) 17.

[26] J. Perdigão, K.N. Jr May, A.D. Jr Wilder, M. Lopes, Op. Dent. 25 (2000) 186.

[27] D.H. Pashley, J.A. Horner, P.D. Brewer, Op. Dent. 5 (1992) 137.

[28] M. Toledano, R. Osorio, E. Osorio, C. Prati, R.M. Carvalho, J. Dent. 33 (2005) 349.

[29] B. Van Meerbeck, G. Willens, J.P. Cellis, J.R. Roos, M. Braem, P. Lambrechts, G. Vanherle, J. Dent. Res. 72 (1993) 1434.

[30] A.O. Dourda, A.J. Moule, W.G. Young, Int. Endod. J. 27 (1994) 184.

[31] H.C. Rushel, O. Chevitarese, J. Clin. Ped. Dent. 26 (2002) 3.

[32] A. Hirayama, M. Yamada, K. Miake, J. Dent. Res. 64 (1985) 743.

[33] N. Nakabayashi, A. Watanabe, K. Igarashi, J. Biomed. Mater. Res. A 68 (2004) 558.

[34] R.M. Carvalho, M. Yoshiyama, E.L. Pashley, D.H. Pashley, Arch. Oral Biol. 4 (1996) 369.

[35] D.H. Pashley, Y. Zhang, R.M. Carvalho, F.A. Rueggeberg, C.M. Russell, J. Dent. Res. 8 (2000) 1579.

[36] F. El Feninat, T.H. Ellis, E. Sacher, I. Stangel, Dent. Mat. 17 (2001) 284.

[37] L. Tjäderhane, H. Larjava, T. Sorsa, V.J. Uitto, M. Larmas, T. Salo, J. Dent. Res. 8 (1998) 1622.

[38] S. Martin de Las Heras, A. Valenzuela, C.M. Overall, Arch. Oral Biol. 45 (2000) 757.

[39] A.J. van Strijp, D.C. Jansen, J. DeGroot, J.M. Ten Cate, V. Everts, Caries Res. 37 (2003) 58.

[40] D.H. Pashley, F.R. Tay, C. Yiu, M. Hashimoto, L. Breshi, R.M. Carvalho, S. Ito, J. Dent. Res. 3 (2004) 216.

[41] A. Mazzoni, D.H. Pashley, Y. Nishitani, L. Breschi, F. Mannello, L. Tjäderhane, M. Toledano, E.L. Pashley, F.R. Tay, Biom. 27 (2006) 4470.

[42] J Hebling; D H Pashley; L Tjäderhane; F R Tay. Chlorhexidine Arrests Subclinical Degradation of Dentin Hybrid Layers in vivo. J Dent Res. 2005; 8:741-746.

[43] M.F. Burrow, U. Nopnakeepong, S. Phrukkanon, Dent. Mat. 18 (2002) 239.

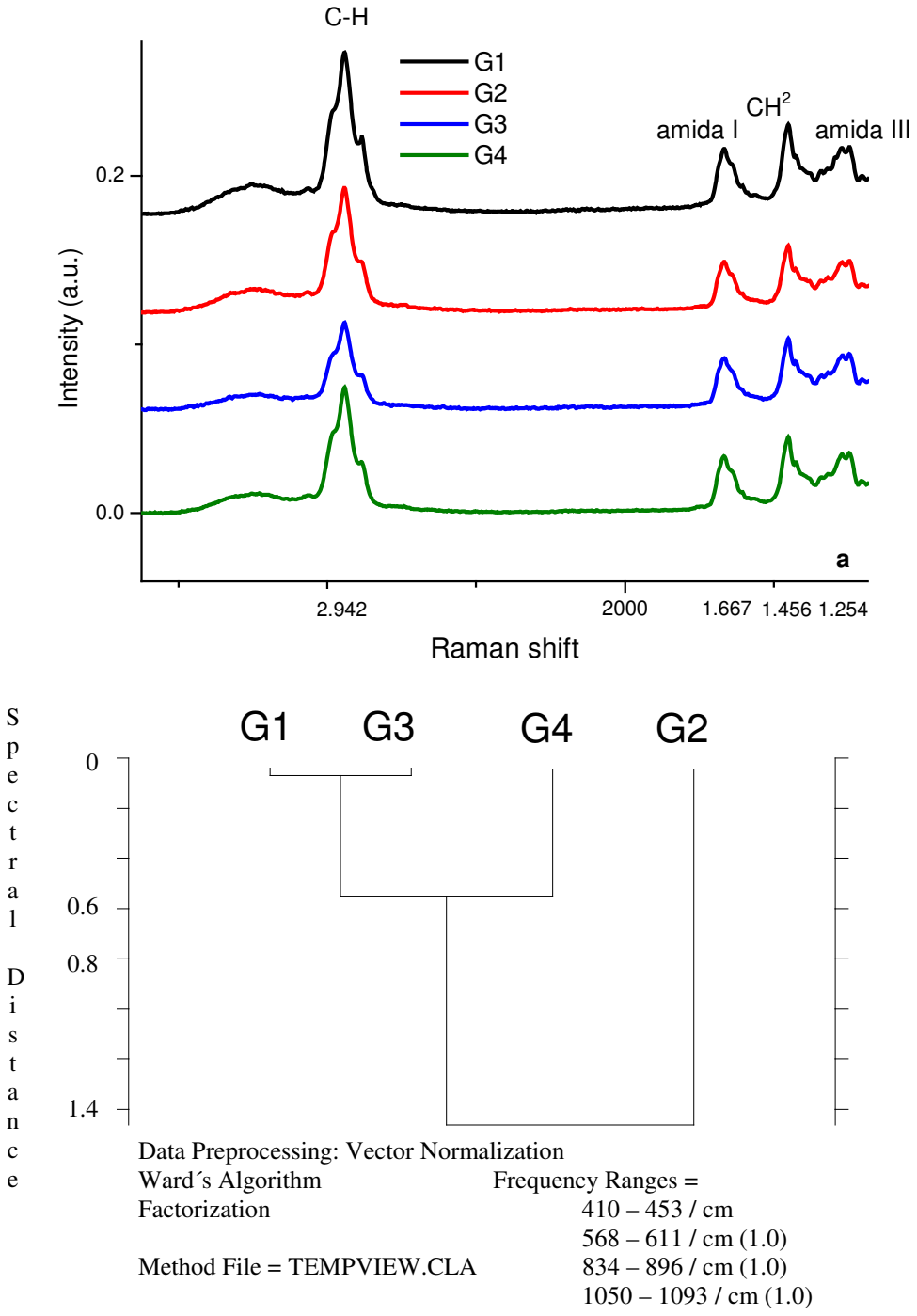
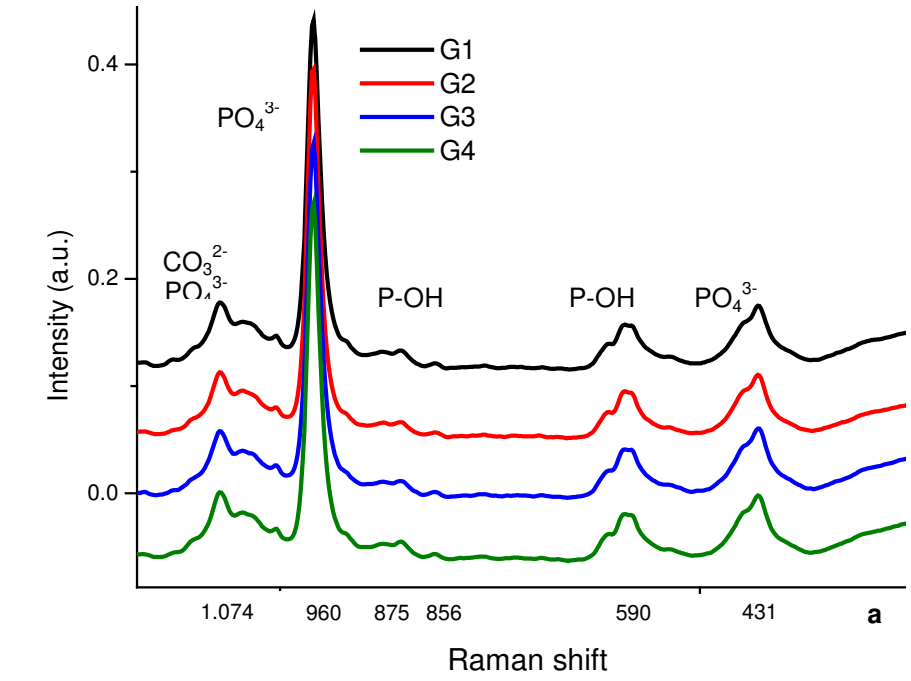


Figure 1 FT-Raman spectra and dendrogram of group means. **a** Organic content spectra with identified peaks of chemical structures (1254, 1456, 1667 and 2942 cm^{-1}). **b** Dendrogram related to spectra showed in fig 1a.



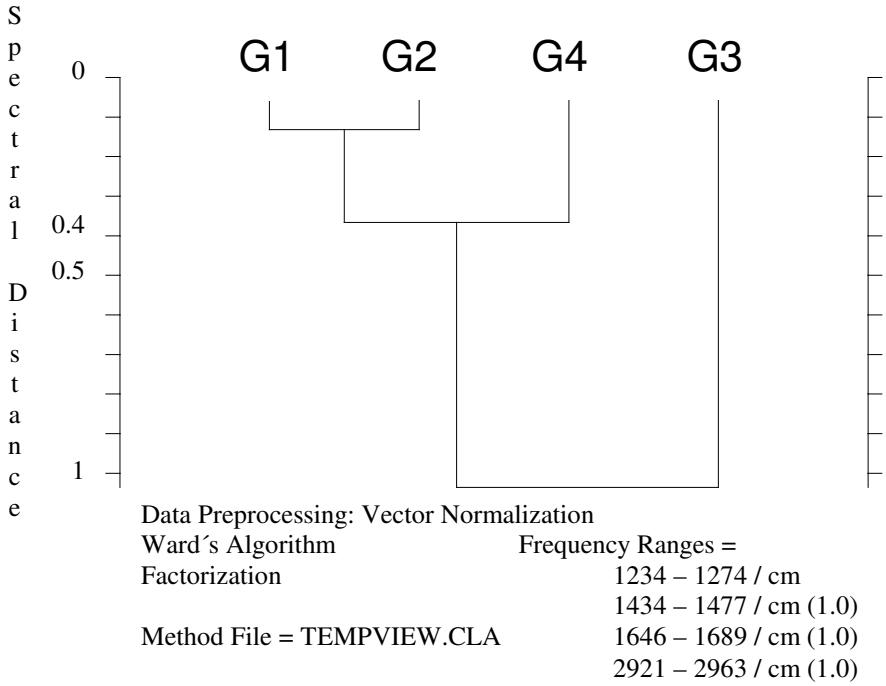


Figure 2 FT-Raman spectra and dendrogram of group means. **a** Inorganic content with identified peaks of chemical structures (431, 590, 875, 856, 960, 1074 cm^{-1}). **b** Dendrogram related to spectra showed in fig 2a.

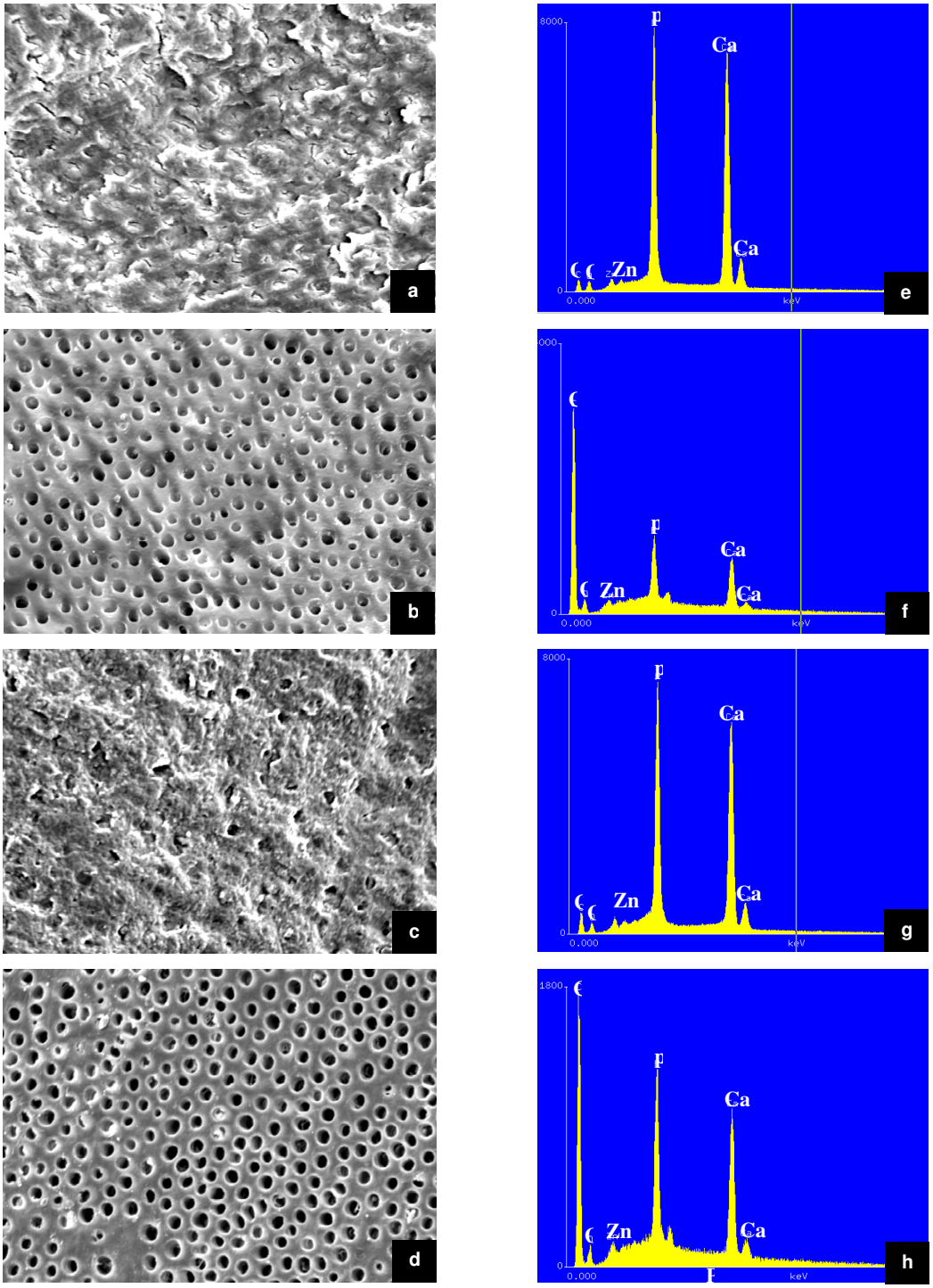


Figure 3 EDS/SEM results of studied groups. Images **a, b, c, d** illustrating the scanned area by EDS related to G1, G2, G3 and G4, respectively. Spectra **e, f, g, h** showing the chemical elements identified related to its scanning areas showed in the fig **a, b, c, d,** respectively.

Table 1 Means and Standard Deviations of Knoop Hardness Test.

Studied Groups	**Means (Standard Deviations)**
G1	45.7 (3.2) a
G2	28.5 (2.8) b
G3	46.2 (3.6) a
G4	27.6 (3.1) b

Different letters indicate significant difference.

3.2 CAPÍTULO 2

"NaOCl modifica o arranjo molecular do conteúdo inorgânico da dentina da câmara pulpar decídua e permanente".

"NaOCl changes the inorganic content molecular arrangement of primary and permanent pulp chamber dentin".

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Running title: **Dentin molecular changes**

Keywords – dentin, FT-Raman, mineral content, sodium hypochlorite.

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ABSTRACT

Aim: The dentin quality of primary and permanent pulp chamber was inspected by Fourier Transformed Raman Spectroscopy (FT-Raman) and Scanning Electron Microscopy (SEM). Fragments of pulp chamber dentin were obtained by mesio-distal section of twenty human molars crowns (primary and permanents). **Methodology:** The fragments were distributed into 8 groups (n = 5): Primary Teeth: G1 - dentin; G2 - dentin irrigated with NaOCl 1% (30 min); G3 - dentin irrigated with NaOCl 1% (30 min) and etched by 35% phosphoric acid; G4 - dentin etched by 35% phosphoric acid. Permanent Teeth: G5 - dentin; G6 - dentin irrigated with NaOCl 1% (30 min); G7 - dentin irrigated with NaOCl 1% (30 min) and etched by 35% phosphoric acid; G8 - dentin etched by 35% phosphoric acid. The spectra were subjected to the Cluster Analysis. The photomicrographs were scored. **Results:** Inorganic Content (IC): There was difference between primary and permanent dentin. G1 and G4 showed similar IC, followed by G2 that showed intermediate IC, not differing from G1 and G2, but higher than the IC of G3. G6 and G7 showed similar IC, followed by G8 that showed intermediate IC, not differing from G6 and G7, but higher than the IC of G5. Organic Content (OC): G7 and G8 showed similar OC; G2 showed intermediate OC; G4 and G6 showed similar OC, as well as, G3 and G5 that, in turn, were quite similar to G1. The photomicrographs reveled different features among G1, G2 and G3, being G4 similar to G3. **Conclusions:** The NaOCl changed the inorganic content in both dentitions; regardless the following phosphoric acid etching, however, nothing was detected morphologically on groups treated with NaOCl and etched.

INTRODUCTION

Resin monomers have been widely used within pulp chamber in order to restore endodontically treated teeth (Chersoni et al., 2005; Salameh et al., 2006; Dallari et al. 2006; Maruoka et al. 2006). Regardless the restoration type be direct or indirect, the principle of adhesion is the same, based on hybridization. The mineral phase should be dissolved causing no damage in the exposed collagen matrix, which is infiltrated by resin monomers that polymerize (Nakabaiashi, 1982). The ideal hybrid layer would be characterized as a three-dimensional collagen-resin biopolymer which provides a continuous and stable link between the bulk of the adhesive and the dentin substrate (Misra et al., 2004).

Nevertheless, the dentin available to adhesion procedure within pulp chamber is covered by predentine and retained pulpal components. There are factors that affect the retention of odontoblasts to predentine, such as the odontoblastic processes, which engage space in the dentinal tubules and are considered an effective physical barrier (Nagaoka et al., 1995); fibronectin attaching cell to extracellular matrices (Yoshida et al., 1995); cell-to-cell junctions between odontoblastic cell bodies (Ikeda & Suda 2002); von Korff fibres extending from the pulp into mineralized dentine (Bishop et al., 1991); and mainly collagen within tubules (Puapichartdumrong, 2005). The predentine is easily removed by action of endodontic instruments and chemical solutions (Evans et al., 2001; Puapichartdumrong et al., 2005). However, the subjacent dentin has not been sufficient studied as a potential substrate to adhesion with resin monomers.

This subjacent dentin of pulp chamber contacts irrigant solutions used in pulp therapy as sodium hypochlorite (NaOCl), which is a root intracanal irrigant widely recommended due to its antibacterial and organic tissue dissolution properties (Czonstkowsky et al., 1990; Tasman et al., 2000). The smear layer produced during endodontic treatment of the dentin from the pulp chamber is not affected by mechanical instrumentation in the same way as the root dentin. Consequently, the irrigant solutions action on dentin surface is considered regular (Ozturk & Ozer, 2004).

Although the NaOCl is useful to remove retained pulpal components, it may decrease the physical and mechanical properties of dentin. Theoretically, the NaOCl breaks down to sodium chloride and oxygen, which could provide oxidation of some components

in the dentin matrix (Yui et al., 2002) and consequently it decreases the elastic modulus and flexural strength of dentin (Sim et al., 2004). Allied to this it could affect the resin penetration into the dentin structure and/or the monomers polymerization in the demineralized dentin, so it could influence the restorations performance quality (Sim et al., 2004).

Some studies have been carried out to evaluate the bond strength of resin materials upon the permanent dentin of pulp chamber previously subjected to pulp therapy with NaOCl irrigation. Regardless adhesive systems (Nikaido et al., 1999; Ozturk & Özer, 2004) or resin luting agents (Ari et al., 2003) tested, these resin materials tended to decrease their bond strength. The lost bond strength can be recovered when NaOCl irrigation was followed by 10% ascorbic acid or 10% sodium ascorbate (Morris et al., 2001). However, a mechanic test can not evaluate the changes caused by NaOCl in dentin structure.

There are no chemical studies about the real changes on molecular structures caused by NaOCl irrigant on the primary dentin. In permanent dentin, the NaOCl changes molecular structures (Tsuda et al. 1996; Driscoll et al., 2002). Therefore, the purpose of this study is to investigate at chemical and morphological levels the pulp chamber dentin after NaOCl irrigation, and followed by etching with phosphoric acid as occurs on clinical practice when endodontically treated teeth had its lateral walls restored with resin materials, in both primary and permanent teeth.

MATERIALS AND METHODS

Selection of teeth

Extracted sound human third molars and primary molars, from subjects requiring such extractions as part of their dental treatment, were selected for this study. These procedures have been conducted according to an informed consent protocol that has been approved by the Research Ethics Committee of FOP/UNICAMP (Approval No. 083/2005) according to the Resolution of the National Commission of Ethics in Research. Ten maxillary first primary molars and 10 third molars were selected to study after inspection that they had no caries, composite restorations or any abnormality confirmed by digital radiographies (Cygnus X- Ray; Cygnus, San Francisco, CA, USA). The third molars

had its roots cut at furcation level. The primary teeth did not present roots due the exfoliation process.

Specimen preparation

The cervical surface of each tooth was fixed with wax on acrylic plates. The teeth were sectioned mesio-distally paralleled to the long axis using a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL, USA). The pulpal components were removed by manual instrument. After that, each part of tooth obtained was individually sonicatted immersed in deionized water inside a Becker for 5 minutes in order to remove any residual impurities. Two parts were obtained from each tooth, totaling 20 parts from primary teeth and 20 parts from permanents teeth, randomized distributed into groups (n=5). The groups of this experiment were:

Primary Teeth

G1 - pulp dentin;

G2 - pulp dentin irrigated with NaOCl 1%* (30 min);

G3 - pulp dentin irrigated with NaOCl 1%* (30 min) and then, etched by 35% phosphoric acid**;

G4 - pulp dentin etched by 35% phosphoric acid**.

Permanent Teeth

G5 - pulp dentin;

G6 - pulp dentin irrigated with NaOCl 1%* (30 min);

G7 - pulp dentin irrigated with NaOCl 1%* (30 min) and then, etched by 35% phosphoric acid**;

G8 - pulp dentin etched by 35% phosphoric acid**.

The 1% NaOCl solution treatment (PRODERMA LTDA, Piracicaba, S.P., Brazil*****) was accomplished in order to simulate irrigation during the pulp therapy. The five parts of chamber dentin selected from to each group, which received the treatment were sonicatted (Maxiclean 750, Unique Ind. e Comp., Indaiatuba, SP, Brazil) with 1% NaOCl solution inside Beckers about 30 min. A Pilot study showed that morphologically there is no difference between this type of procedure compared to one accomplished by Borges et al., 2005, in which dentin parts were placed in a plastic recipient while the solution flush

out through a disposable pipette during 30 min simultaneously with vibration using the Multi-Sonic-s ultrasound (Gnatus, Ribeirão Preto, S.P., Brazil).

The 35% phosphoric acid (pH 0.6) was manipulated in liquid form by Biochemical Laboratory of Piracicaba School of Dentistry, UNICAMP, São Paulo, Brazil**, in order to avoid that the colloidal silica present in the phosphoric acid gel form could prejudice the spectroscopy measurements and the photomicrography's as shown by a pilot test (unpublished data). The specimens were etched for 15 s and rinsed with tapered water for 10 s. Each specimen was stored immersed into Karnovisk solution in order to fix and to prevent the exposing collagen of dentin until the molecular analysis.

Fourier Transformed Raman Spectroscopy (FT-Raman)

The molecular measurements were carried out on FT-Raman Spectrometer RFS 100 (Bruker Optics Inc, Billerica, MA, USA) equipped with OPUS software (4.2 version, Copyright © Bruker Optics GmHb 1997-2002, Billerica, MA, USA) at University Vale do Paraíba (UNIVAP, São José dos Campos, SP, Brazil).

The spectra were recorded at a 4 cm^{-1} spectral resolution of 7 mm laser beam aperture by the use of a focused 1064 ηm line. The Nd:YAG near-IR laser was used as an excitation line. The maximum laser power incident on the specimens was ~ 360 mW. The specimens were examined upon sampling plate fixed by double face adhesive tape, perpendicular to the laser beam. One measurement was carried out upon the middle flat dentin with 1 mm^2 scanning area and ~ 6 min integration time, at room temperature.

Scanning Electronic Microscopy (SEM)

All samples analyzed by FT-Raman were prepared according to Perdigão et al., 1995 protocol of dentin sample fixation and dehydration. Then, they were gold sputtered on a Denton Vacuum, Desk II (Buffallo, NJ, USA). Micrographs (x2000 magnification) were obtained by Scanning Electron Microscopic JEOL JSM-5600LV (Tokyo, Japan) operating at 15 kV, with 15 mm of work distance and spotsize 38.

Each image was examined by an experienced examiner on dentin photomicrographs analysis, in a double-blind designer, according to pre-established scores (table 1). Two measurements with a 1 month interval were performed by the examiner to reach the intra-examiner calibration. The Person Coefficient showed 99% of reliability. The images were classified according to the established scores on table 1.

Statistical Analysis

To verify intra-specimen variation, FT-Raman spectrum was recorded in three different regions in two specimens of each group. It was observed that the spectral patterns were exactly the same and there were no difference after Cluster Analysis application (see explanation below) and thereafter it was established that one measurement for each specimen was legitimate. The entire spectrum in the range ~ 400 to 3000 cm^{-1} was selected since it included the main bands related to both organic and inorganic structures of dentin.

In this study, were analyzed forty Raman spectra of human dentin divided into eight groups. Cluster analysis was performed using data pretreatment techniques, that is a combination of smaller regions of the organic and inorganic regions of the dentin Raman spectra, which were most discriminative to achieve optimal classifications. The bands are a set of wavenumbers which contains a main wavenumber related to the peak of the band. The wavenumbers of the peaks were related to the molecules of dentin and confirmed by international literature [12, 14-15]. The peaks used in this study were related to the Principle Components Identified (PCIs). The peaks of the organic content were: 1254, 1456, 1667, 2942 cm^{-1} related to PCIs C-H bonds, amide I, CH^2 bonds, amide III, and for the inorganic content the following peaks: 431, 590, 856, 875, 960, 1074 cm^{-1} related to PCIs CO_3^{2-}, $PO_4^{3-,}$ P-OH, PO_4^{3-} bonds. The spectra of each group were composed by 727 data points in the spectral range 400 to 1800 cm^{-1} and were divided into organic and inorganic spectra. The organic bands, assigned in this study, were chosen at 1234-1274, 1434-1477, 1646-1689, 2921-2963 cm^{-1}, and the inorganic bands at 410-453, 568-611, 834-896, 938-981, 1050-1093 cm^{-1}, providing approximately 22 data points to each assigned band. Principle Components Analysis was used as patterns detection technique to reduce the organic and inorganic bands data for improvement of the discrimination capacity of the software (OPUS software (4.2 version, Copyright © Bruker Optics GmHb 1997-2002, Billerica, MA, USA). Clustering techniques of the individual spectrum were applied to achieve groups according to spectral similarity. Cluster analysis was performed using the all principal components identified. To organic content, the spectrum collect was since the wavenumber of the C-H band beginning until the wavenumber of amida III band end. To inorganic content, the spectrum collect was since the wavenumber of the CO_3^{2-}, PO_4^{3-} band

beginning until the wavenumber of the other PO_4^{3-} band end. All spectra ranges used were equally weighted, and Average Linkage algorithm was applied. For calculating the distance matrix, Factorization was used. For a better visualization of all analysis, dendrogram of hierarchical cluster analysis were performed.

RESULTS

In this study, the mean spectra of the groups have the same baseline; however, they were dissociated in order to improve the visualization of each spectrum (fig 1a, fig 2a). The spectrum features were very similar among groups with no visible broadening of any band (fig 1a, fig 2a). However, the statistical analysis showed that there were differences among groups as can be observed by the dendrograms, for both organic and inorganic content. Dendrograms are formed by principal root that is dissociated into right and left sides. When groups are in the same side but present different spectral distances in the baseline among them, they are statistically similar. However, when groups are in the same side and present the same spectral distance in the baseline they are highly similar among them. In opposite, when groups are into different sides within keys with different spectral distance values, they are statistically different among them.

Figure 1b shows that G5, G6, G7 and G8 are at the right side of the principle root. It means that they were different from G1, G2, G3 and G4 that were at the left side of the principle root. Therefore, primary dentin presented its mineral content molecular arrangement different from those of permanent dentin. Regarding primary dentin, G1 and G4 showed the same spectral distance (near to 0.05), with 0.1 spectral distance difference to G2 (with spectral distance near to 0.15), that, in turn, showed 0.1 spectral distance difference to G3 (with spectral distance near to 0.25).

Figure 2b shows the results of organic content. G1, G3, G5, G4, G6 are at the right side of the principle root. It means that they were different from G2, G7 and G8 that were at the left side of the principle root. G3 and G5 showed the same spectral distance (near to 0), as well as G4 and G6. However, G3 and G5 were next to G1, which means that despite G1 present different spectral distance (~ 0.02) it was more similar to G3 and G5 than G4 and G6. On the other hand, G7 and G8 showed the same spectral distance (~ 0.35), and 0.05 spectral distance different from G2 (with spectral distance near to 0.4).

The photomicrographs showed different features according to each study protocol, as follows: G1, G2, G3 and G4 were classified with scores A, B, and C respectively (Table 1).

DISCUSSION

The Cluster Analysis, supported by Principal Components Analysis (PCA) among groups is a reliable method of evaluating the FT-Raman spectrum because it can show significant differences that are difficult to be seen only through spectral exam. In general, spectroscopic studies analyze the bands width. For example, if a peak is thin the molecule is completely formed; conversely, if a peak is broad changes can occur in the molecule structure (Spencer et al., 2001; Tusda & Arends, 1998; Penel et al., 1998). In this study, the spectra did not show visible differences among the groups. The limitation of this study was the 20 μm laser penetration depth. This depth included the predentine, the retained pulpal components and the subjacent dentin available to adhesion. However, the Cluster Analysis accuracy showed that there were changes in both organic and inorganic content according to the treatments studied.

Inorganic content

The most evident difference among the groups was the noticeable difference between primary and permanent dentin pulp chamber related to inorganic content. This result corroborates with data obtained by middle dentin of crowns (personal data) that showed different molecular arrangement of the inorganic content in primary dentin when compared to the inorganic content of permanent dentin. Primary tooth is a substrate prepared to have short period of function in mouth and probably, the chemical reactions of its mineral content might be higher than permanent one. The aging process increased apatite crystallinity (Freeman et al., 2001; Tesch et al., 2001), providing the hardness property of primary dentin similar to the permanent dentin (unpublished data). Although primary dentin is lesser mineralized than permanent one, the increase of apatite crystallinity along time can offset this difference providing similar mechanical strength to the old primary teeth.

The 1% NaOCl during 30 min simulated a clinic section of endodontic therapy. It was sufficient to modify the molecular arrangement of inorganic content, regardless if

followed by etching with phosphoric acid or not. Some studies have been shown the effects of NaOCl on dentin (Czonstkowsky et al., 1990; Tasman et al., 2000). The NaOCl irrigation reduced the bond strength of 4 adhesive systems to pulp chamber mesial walls and to root canal dentin (Ozturk et al., 2004; Ari et al., 2003). However, the decrease of bond strength caused by NaOCl was recovered by application of ascorbic acid or/and sodium ascorbate (Morris et al., 2001; Yui et al., 2002). Some authors argue that sodium hypochlorite breaks down to sodium chloride and oxygen, that provides oxidation of some components in the dentin matrix (Tsuda et al., 1996; Morris et al., 2001; Yui et al., 2002) and consequently decreases the elastic modulus and flexural strength of dentin (Grigoratos et al., 2001; Sim et al., 2001). In addition it could be critical for the interfacial initiation of resin monomers polymerization in the demineralized dentin and could prejudice the restorations quality (Nikaido et al., 1999; Perdigão et al., 2000; Sim et al., 2001).

It seems that the chemical changes detected in the inorganic content of this current study can be result of some calcium phosphate and calcium carbonate molecules reaction with sodium hypochlorite, as follows, respectively: $6Na(ClO) + Ca^{+2}{}_3\ (PO^{-3}{}_4)_2 = 2Na_3PO_4 + 3Ca(ClO)_2$ and $2Na(ClO) + CaCO_3 = Na_2CO_3 + Ca(ClO)_2$. If some hydroxyapatite molecules could react with sodium hypochlorite, the result would be calcium hypochlorite, sodium phosphate and water, which also could contribute to molecular changes detected by FT-Raman. These reaction could solubilize some mineral molecules, leading a slow dissolution of encapsulated collagen of dentin, the so called "organic 2" component that covers the hydroxyapatite nanocrystals and is 2-5 nm thick (Brik et al., 2000). This chemical change could produce unbound hydroxyapatite crystals (Di Renzo et al., 2001; Fattibene et al., 2005), and revel a mineral surface rich in hydroxyl, carbonate and phosphate groups as seen by Di Renzo et al., (2001). The exposition of unbound hydroxyapatite crystals by NaOCl irrigation could increase the area of adhesion of dentin after etching. Would be the resin monomers able to penetrate within a whole of this area? It is known that one of the problems of these adhesive systems is the lack of resin monomers to reach all depth of etched dentin area (Nakabaiashi et al., 1998). On the other hand, the hydroxyl, phosphate and also carbonate groups can be available for chemical bonding when used adhesives systems that contains acidic monomers (Moszner et al.,

2005). Further studies are needed to clarify the interactions of self-etching adhesive systems with the dentin treated with NaOCl.

Organic content

This current study showed that organic content become similar to all groups. Neither NaOCl nor phosphoric acid divided sample due their action. The primary pulp chamber dentin (G1) showed similarities to primary dentin treated with NaOCl (G3) that, in turn, was highly similar to permanent pulp chamber dentin (G5). The primary pulp chamber dentin (G4) etched by phosphoric acid was highly similar to permanent dentin treated with NaOCl (G6). The G2, primary pulp chamber dentin treated with NaOCl, showed fewer similarities with the other groups. Finally, two permanent groups were highly similar each others, that are, pulp chamber dentin treated with NaOCl followed by etching (G7) and pulp chamber dentin only etched with phosphoric acid (G8).

The changes of organic content caused by NaOCl can be understood as due to the loss of protein and water (Driscool et al. 2002). A chemo mechanical approach which includes mainly NaOCl in its composition (Carisolv®), removed all organic compounds of caries lesion, providing the reminiscent dentin chemically similar to sound dentin (Arvidsson et al., 2002).

The acid etching also reacts with organic components of dentin contributing to the mix of primary and permanent organic content arrangement of dentin. The phosphoric acid partially solubiblizes acidic noncollagenous phosphoproteins, glycosaminoglycans (GAGs) and low molecular weight peptides, including solubilized low molecular weight collagen (Nakabayashi et al., 2004). In addition, the 35% phosphoric acid for 15 s broadened the amides I and III peaks in contrast to the dentin totally demineralized by EDTA, which preserved their amides molecules (Spencer et al., 2001). This concern suggest that the phosphoric acid could modify organic components of collagen molecule, but not highly as a solvent for the organic portion as stated by Spencer et al., (2001).

The components of Karnovisk solution (glutaraldehyde and phosphate-buffered saline) can stiffen the acid etched demineralized dentin matrix (Pashley, et al., 2000) and can contribute to the chemical changes if some alteration occurred with triple helices of collagen by Karnovisk solution interaction. Conversely, the matrix metalloproteinases (MMPs), which are within the mineralized dentin, with collagenolytic and gelatinolytic

activities (MMP-2 and MMP-20), can be released and activated by low pH, leading collagen degradation (Tjäderhane et al., 1998; Martin de Las Heras et al., 2000; van Strijp et al., 2003). In this study it could be another factor to organic content changes. The 37% phosphoric acid gel etching for 15 s decreased its intrinsic mineralized dentin powder collagenolytic activity because its low pH, which could denaturates the MMPs or inactivates it by calcium ions removal (Pashley et al., 2004). Despite of this fact, the concern about the action of phosphoric acid at concentrations commonly used in adhesive bonding procedures has been still. There is dentin collagen hydrolytic degradation with no bacterial colonization over time in aqueous environment (Pashley et al., 2004). Moreover, the simplified etch-and-rinse adhesives can reactivate endogenous enzymatic activities in dentin previously inactivated by phosphoric acid-etching (Mazzoni et al., 2006).

Morphological analysis

In this study, the teeth had its pulpal components removed by manual instrument. This pulp mechanical separation was not effective to remove the pulpal components, odontoblastic layer and predentine (figs 3a and 4a) in agreement with Chadha & Bishop, 1996, even followed by sonication with deionizated water for 5 minutes. A previous study showed there were no differences between morphological patterns of predentine and residual pulpal debris (Evans et al., 2001). However, in this study, the photomicrographs of pulp chamber dentin without any kind of treatment, regardless primary or permanent, were considered with presence of retained pulpal components and possible odontoblastic layer and predentine.

There was no difference between primary or permanent pulp chamber dentin. There were differences according to each treatment. G1 and G2 were classified with scores A and B, respectively, while G3 and G4 received the score C (Table 1). The photomicrographs showed that the retained pulpal components upon dentin surface (figs. 3a and 4a) were completely removed with 1% NaOCl irrigation during 30 min (figs 3b and 4b) as observed by Evans et al. (2001). However, the concavities and convexities (figs. 3b and 4b, respectively) from irregular dentin area and a roughness due to mineral structure were maintained. When the irrigation was followed by phosphoric acid etching (figs. 3c and 4c), it could be observed that the concavities, convexities and roughness of dentin were lost and the surface become smooth. Furthermore, the tubules orifices were enlarged, the dentin

within tubules was disclosing and the thickness of intertubular dentin was reduced, in agreement with Torabinejad et al., (2003) that found the same features in dentin surface subjected to NaOCl irrigation followed by use of MTAD (a mixture of a tetracycline isomer, an acid, and a detergent). The MTAD is an acidic solution with a pH of 2.15 that is capable of removing inorganic substances. The Figs. 3d and 4d represent the dentin only etched with phosphoric acid, showing the enlarged tubules lumen and decreased of intertubular dentin thickness as also observed in Figs. 3c and 4c. According to Marshall et al., (2001) the deproteinization alone does not open the channels in mineralized dentin because the mineral protect much of protein and the etching alone not completely open the channels since they remain filled with protein. However, morphological differences were not observed among the combined steps of etching and NaOCl, or only acid etching. The changes of inorganic content detected by FT-Raman are not visible by MEV analysis.

CONCLUSIONS

Within the limitations of this study it can be concluded that:

1. The 1% NaOCl changes the inorganic content arrangement of primary and permanent dentin pulp chamber;
2. The photomicrographs did not detect the chemical changes caused by NaOCl when followed by phosphoric acid etching.

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REFERENCES

Ari H, Yasar E, Belli S (2003) Effects of NaOCl on bond strengths of resin cements to root canal dentin. *Journal of Endodontics* 29, 248-51.

Arvidsson A, Liedberg B, Kenneth M, Lyvén B, Sellén A, Wennerberg A. (2002) Chemical and topographical analyses of dentine surfaces after Carisolv treatment. *Journal of Dentistry* 30, 67-75.

Borges AFS, Correr GM, Sinhoreti MAC, Consani S, Sobrinho LC, Puppin Rontani RM (2006) Compressive strength recovery by composite onlays in primary teeth. Substrate treatment and luting agent effects. *Journal of Dentistry* 34, 478-484.

Brik A, Haskell E, Brik V, Scherbina O, Atamanenko O (2000) Anisotropy effects of EPR signals and mechanisms of mass transfer in tooth enamel and bones. *Applied Radiatastic Isotropy* 52, 1077-83.

Chada S, Bishop MA (1991) Effect of mechanical removal of the pulp upon the retention of odontoblasts around the pulp chamber of human third molars. *Archives of Oral Biology* 41, 905-909.

Chersoni S, Acquaviva GL, Prati C, Ferrari M, Grandini S, Pashley DH et al. (2005) In vivo fluid movement through dentin adhesives in endodontically treated teeth. *Journal of Dental Research* 84, 223-7.

Czonstkowsky M, Wilson EG, Holstein FA (1990) The smear layer in endodontics. *Dental Clinic of North American* 34, 13-25.

Dallari A, Rovatti L, Dalari B, Mason PN, Suh BI (2006) Translucent quartz-fiber post luted in vivo with self-curing composite cement: case report and microscopic examination at a two-year clinical follow-up. *Journal of Adhesive Dentistry* 8, 189-95.

Di Renzo M, Ellis TH, Sacher E, Stangel I (2001) A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteinization. *Biomedical* 22, 793-797.

Driscoll CO, Dowker SEP, Anderson P, Wilson RM, Gulabivala K (2002) Effects of sodium Hypochlorite on root dentine composition. *Journal Materials Science Materials Medicine* 13, 219-223.

Evans GE, Speight PM, Gulabivala K (2001) The influence of preparation technique and sodium hypochlorite on removal of pulp and predentine from root canals of posterior teeth. *International Endodontic Journal* 34, 322-330.

Fattibene P, Carosi A, De Coste V, Nucara A, Postorino P, Dore P (2005) A comparative EPR, infrared and Raman study of natural and deproteinated tooth enamel and dentin. *Physical Medical Biology* 50: 1095-1108.

Freeman JJ, Wopenka B, Silva MJ, Pateris JD (2001). Raman spectroscopic detection of changes in bioapatite in mouse femora as fuction of age and in vitro fluoride treatment. *Calcified Tissue International* 68, 156-162.

Grigoratos D, Knowles J, Ng Y-L, Gulabivala K (2001) Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *International Endodontic Journal* 34, 113-119.

Ikeda H & Suda H (2002) Gap junction between human odontoblasts and other pulpal cells. In*:* Ishikawa T, Takahashi K, Maeda T, eds. Proceedings of the International Conference on dentin/pulp complex; pp. 146-147. Tokyo, Japan: Quintessence.

Marshall GW, Yücel N, Balloch M, Kinney JH, Habelitz S, Marshall SJ. (2001) Sodium hypochlorite alterations of dentin and dentin collagen. *Surface Science* 491, 444-455.

Martin de Las Heras S, Valenzuela A, Overall CM (2000) The matrix metalloproteinases gelatinase A in human dentine. *Archives of Oral Biology* 45, 757-765.

Maruoka R, Nikaido T, Ikeda M, Ishizuka T, Foxton RM, Tagami J (2006) Coronal leakage inhibition in endodontically treated teeth using resin-coating technique. *Dental Materials Journal* 25, 97-103.

Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M et al. (2006) Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomedical* 27, 4470–4476.

Misra A, Spencer P, Marangos O, Wang Y, Katz J (2004) Micromechanical analysis of dentin/adhesive interface by the finite element method. *Journal of Biomedical Materials Research B Applied Biomaterials* 15, 56-65.

Morris MD, Lee KW, Agee A, Bouillaguet S, Pashley DH (2001) Effects of sodium hypochlorite and RC-Prep on bond strengths of resin cement to endodôntica surfaces. *Journal of Endodontics* 27, 753-757.

Moszner N, Salz U, Zimmermann J (2005) Chemical aspects of self-etching enamel-dentin adhesives. *Dental Materials* 21, 895-910.

Nagaoka S, Miyazaki Y, Liu HJ, Iwamoto Y, Kawagoe M (1995) Bacterial invasion into dentinal tubules of human vital and nonvital teeth. *Journal Endodontic Journal* 21, 70-73.

Nakabaiashi N, Watanabe A, Arao T (1998) A tensile test to facilitate identification of defects in resin-bonded dentin specimens *Journal of Dentistry* 26, 379-385.

Nakabayashi N, Kojima K, Masuhara E (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates. *Journal of Biomedical Materials Research* 16, 265-73.

Nakabayashi N, Watanabe A, Igarashi K (2004) AFM observation of collapse and expansion of phosphoric acid- demineralized dentin. *Journal of Biomedical Materials Research* 68A, 558-565.

Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J (1999) Bond strengths to endodontically-treated teeth. *American Journal of Dentistry* 12, 177-80.

Ozturk B, Ozer F, Belli S (2004) An in vitro comparison of adhesive systems to seal pulp chamber walls. *International Endodontic Journal* 37, 297-306.

Pashley DH, Tay FR, Yiu C, Hashimoto M, Breshi L, Carvalho RM et al. (2004) Collagen degradation by host-derivated enzymes during aging. *Journal of Dental Research* 3, 216-221.

Pashley DH, Zhang Y, Carvalho RM, Rueggeberg FA, Russell CM (2000) H^+-induced tension development in demineralized dentin matrix. *Journal of Dental Research* 8, 1579-1583.

Penel G, Leroy G, Rey C, Bres E (1998) MicroRaman spectral study of the PO_4 and CO_3 vibrational modes in synthetic and biological apatites *Calcified Tissue International* 63, 475-481.

Perdigão J, Jr May KN, Jr Wilder AD, Lopes M (2000) The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer. *Operative Dentistry* 25, 186-194.

Perdigão J, Lambrechts P, Van Meerbeek B, Vaherle G, Lopes AL (1995) Field emission ESM comparison of four post fixation drying techniques for human dentin. *Journal Biomedical Materials Research* 29, 1111-20.

Puapichartdumrong P, Ikeda H, Suda H (2005) Influence of the pulpal components on human dentine permeability in vitro. *International Endodontic Journal* 38, 152-159.

Salameh Z, Sorrentino R, Papacchini F, Ounsi HF, Tashikand E, Goracci C et al. (2006) Fracture resistance and failure patterns of endodontically treated mandibular molars restored using resin composite with or without translucent glass fiber posts. *Journal of Endodontics* 32, 752-5.

Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K (2004) Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *International Endodontic Journal* 34, 120-32.

Spencer P, Wang Y, Walker MP, Swafford JR (2001). Molecular structure of acid-etched dentin smear layers-in situ study. *Journal Dental Research* 80, 1802-1807.

Tantbirojn D, Versluis A, Cheng Y, Douglas WH (2003). Fracture toughness and microhardness of a composite: do they correlate? *Journal of Dentistry* 31, 89-95.

Tasman F, Cehreli ZC, Ogan C and Etikan I (2000) Surface tension of root canal irrigants. *Journal of Endodontics* 26, 586-7.

Tesh W, Eidelman N, Roschger P, Goldenberg F, Klaushofer K, Fratzi P. (2001) Graded microstructure and mechanical properties of human crown dentin. *Calcified Tissue International* 69, 147-157.

Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *Journal of Dental Research* 8, 1622-1629.

Tsuda H, Arends J (1994) Orientational micro-Raman spectroscopy on hydroxyapatite single crystals and human enamel crystallites *Journal of Dental Research* 73, 1703-1710.

Tsuda H, Ruben J, Arends J (1996) Raman spectra of human dentin mineral. *European Journal of Oral Science* 104, 123-131.

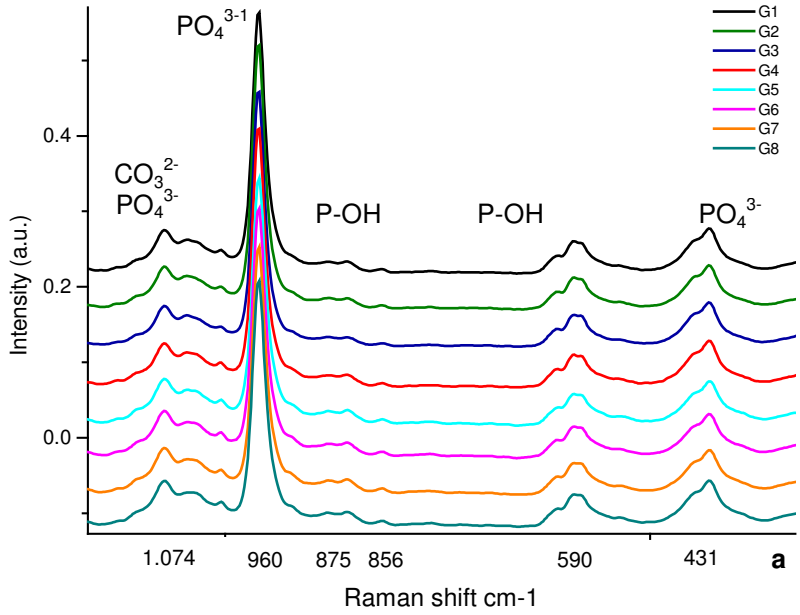
Van Strijp AJ, Jansen DC, DeGroot J, Ten Cate JM, Everts V (2003) Host-derivated proteinases and degradation of dentine collagen in situ. *Caries Research* 37, 58-65.

Yoshiba N, Yoshiba K, Nakamura H, Iwaku M, Ozawa H (1995) Imunoelectron-microscope study of the localization of fibronectin in the odontoblast layer of human teeth. *Archives of Oral Biology* 40, 83-89.

Yui CKY, García-Godoy F, Tay FR, Pashley DH, Imazato S, King NM et al. (2002) A nanoleakage perspective on bonding to oxidized dentin. *Biomedical and Bioengineering* 81, 628-632.

Table 1 Scores employed to classify the photomicrographs.

A	Surface recovered by a visible layer retained pulp components
B	Surface without layer, presence of opened tubules and roughness aspect.
C	Surface without layer, opened and enlarged tubules, decreased thickness of intertubular dentin and some areas with visible collagen.



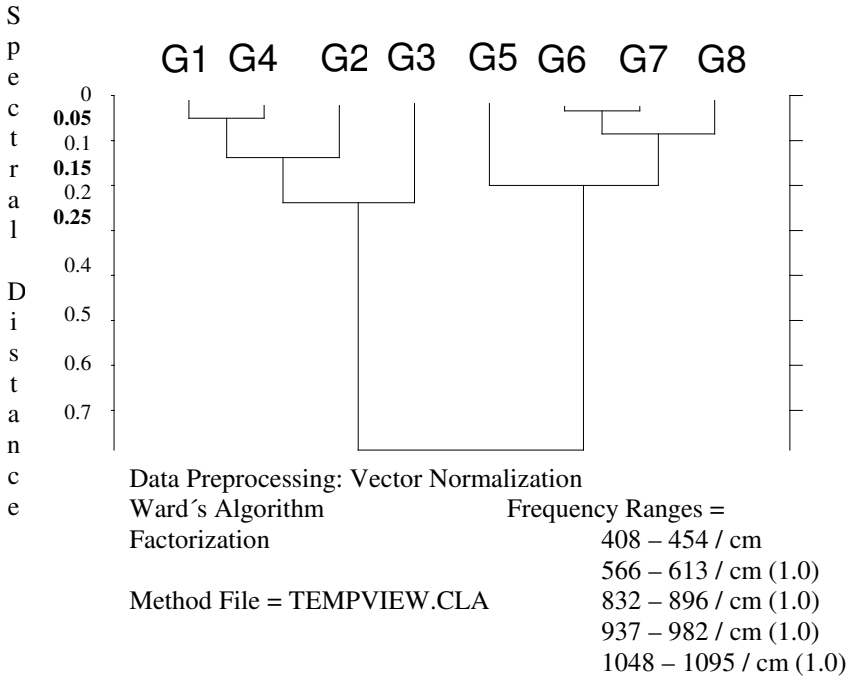
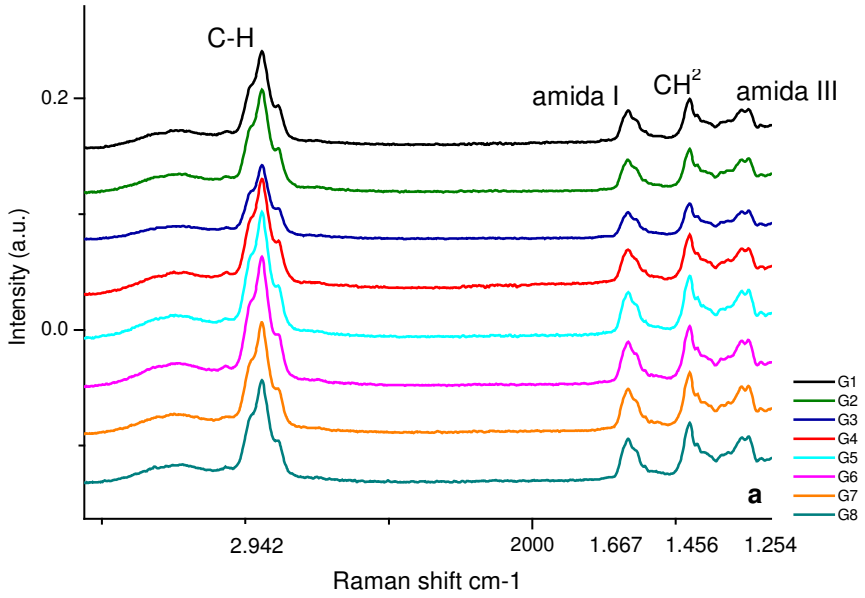
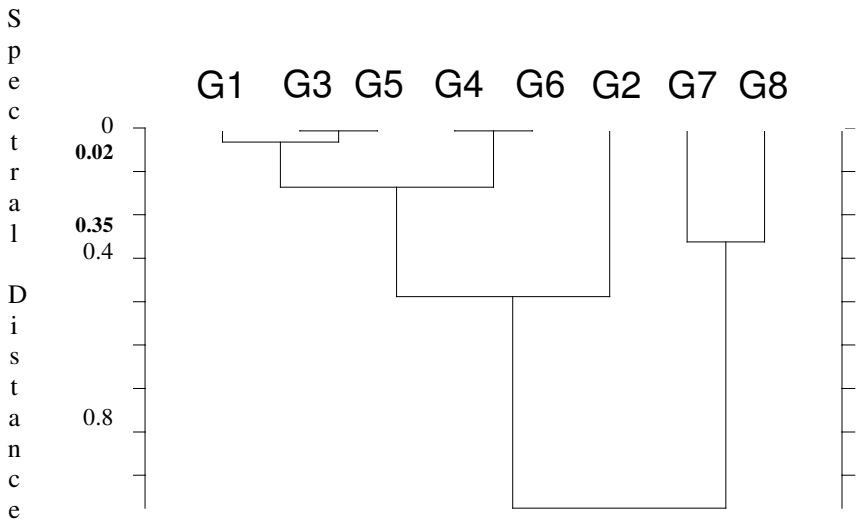


Figure 1 Spectros and dendrograms of group means. **a** Inorganic content with identified peaks of chemical structures (431, 590, 875, 856, 960, 1074 cm^{-1}). **b** Dendrogram related to spectros of 1a.





Data Preprocessing: Vector Normalization
Ward´s Algorithm
Factorization

Method File = TEMPVIEW.CLA

Frequency Ranges =
1234 – 1274 / cm
1430 – 1478 / cm (1.0)
1642 – 1692 / cm (1.0)
2921 – 2963 / cm (1.0)

Figure 2 Spectros and dendrograms of group means. **a** Organic content spectros with identified peaks of chemical structures (1254, 1456, 1667 and 2942 cm^{-1}). **b** Dendrogram related to spectros of figure 2a.

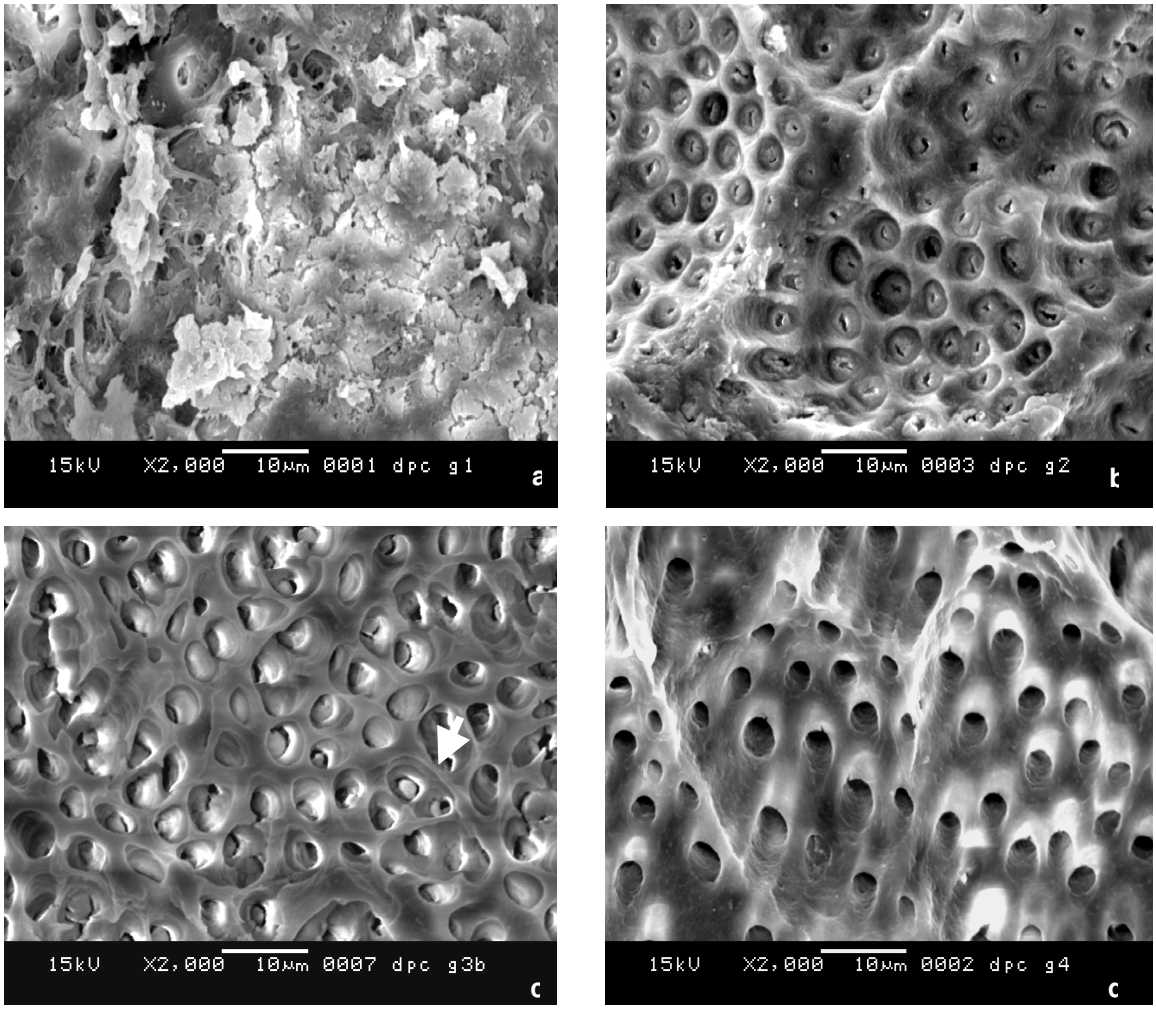


Figure 3 Photomicrographs of primary pulp chamber dentin. All images were carried out with 15 kw, 2000 of magnification and 20 mm of work distance. a – features of pulp chamber dentin. b - pulp chamber dentin treated with 30 min 1% NaOCl. c - pulp chamber dentin treated with 30 min 1% NaOCl followed by etching with 35% phosphoric acid. The white arrow shows the decreased thickness of intertubular dentin in G3. d - pulp chamber dentin etched with 35% phosphoric acid.

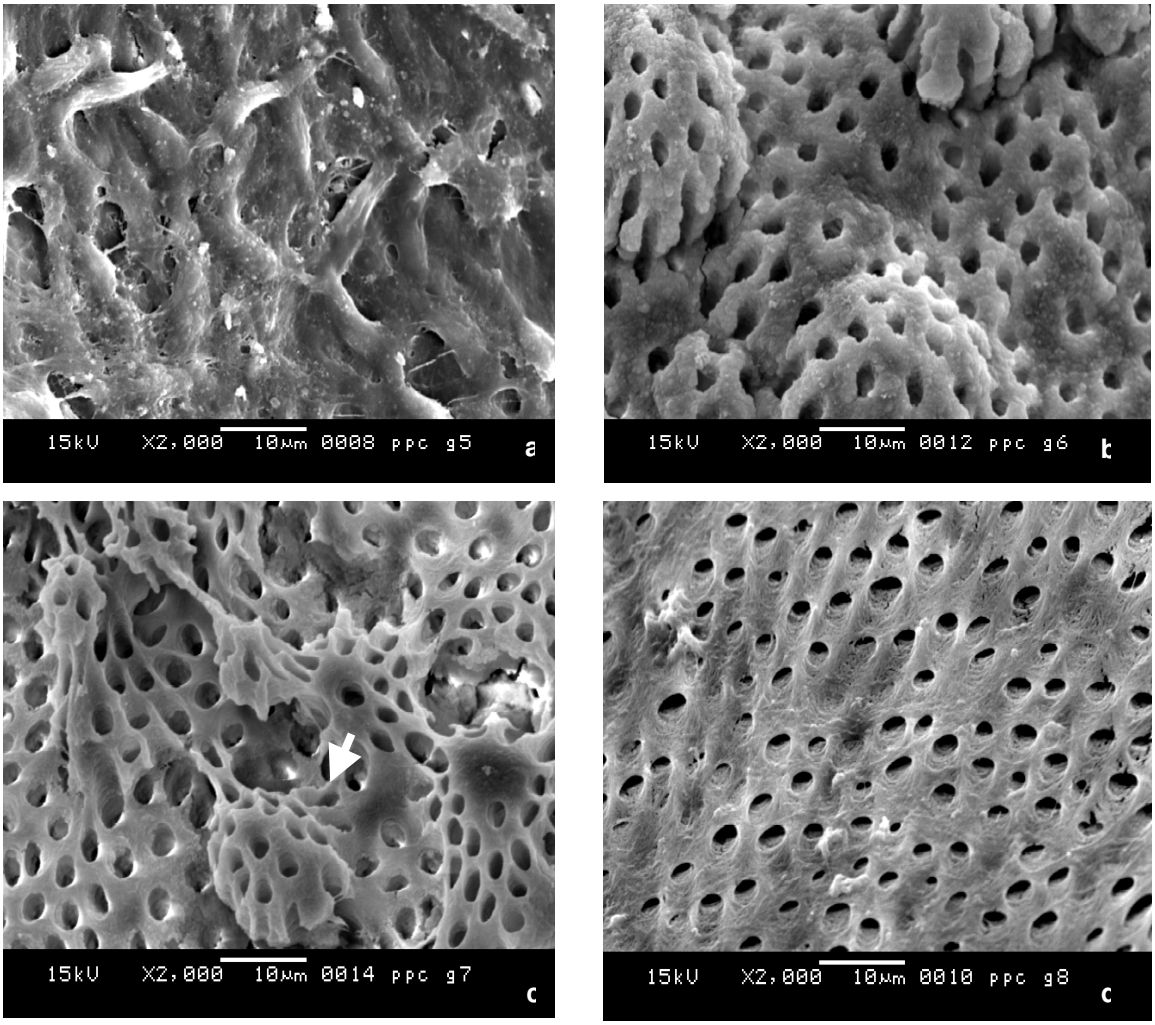


Figure 4. Photomicrographs of permanent dentin pulp chamber. All images were carried out with 15 kw, 2000 of magnification and 20 mm of work distance. a – features of pulp chamber dentin. b - pulp chamber dentin treated with 30 min 1% NaOCl. c - pulp chamber dentin treated with 30 min 1% NaOCl followed by etching with 35% phosphoric acid. The white arrow shows the decreased thickness of intertubular dentin in G7. d - pulp chamber dentin etched with 35% phosphoric acid.

4. CONSIDERAÇÕES GERAIS

Esta tese teve como propósito analisar o conteúdo químico da dentina decídua e permanente para compará-las qualitativamente, bem como após o contato com agente desmineralizante na região média da coroa e após o contato com solução desproteinizante, seguido ou não do agente desmineralizante, na dentina das paredes laterais da câmara pulpar.

No Capítulo 1, verificou-se que não houve diferença entre o conteúdo orgânico da dentina na região média das coroas decíduas e permanentes antes ou após o condicionamento com ácido fosfórico a 35%. No entanto, o conteúdo inorgânico apresentou diferença entre a dentina decídua e a permanente, independentemente se houve ou não condicionamento ácido. O ensaio de dureza revelou que os valores foram similares entre a dentina decídua e a permanente, tanto antes como após o condicionamento ácido. Vale lembrar que o conteúdo inorgânico é o principal responsável por conferir resistência mecânica à estrutura dental, mas não apenas por si, seu arranjo molecular apresenta um papel fundamental nas propriedades mecânicas. É sabido que as moléculas de hidroxiapatita são organizadas de forma cristalina, mais especificamente em geometria hexagonal (Fremann et al. 2001; Tesh et al., 2001). A diferença do arranjo molecular do conteúdo mineral pode ser atribuída à idade dentária, pois os dentes decíduos estudados tinham suas raízes reabsorvidas até o terço cervical, sendo, portanto dentes considerados velhos comparativamente aos terceiros molares permanentes. Fremann et al. (2001) verificaram que a hidroxiapatita com o passar do tempo pode se tornar mais cristalina, fato que confere mais resistência mecânica ao conjunto ao qual ela compõe, fato este que acreditamos ter contribuído para a ausência de diferença quanto à dureza entre dentina decídua e permanente.

A diferença entre a qualidade do conteúdo mineral dentinário decíduo e permanente é consistente, já que no Capítulo 2 observou-se esta diferença na dentina das paredes laterais da câmara pulpar, independentemente do tratamento aplicado a estas superfícies (NaOCl, NaOCl seguido de ácido fosfórico, somente ácido fosfórico). A ação do hipoclorito de sódio resultou na modificação do arranjo molecular do conteúdo inorgânico tanto no substrato decíduo quanto no permanente, independente da aplicação subseqüente do ácido fosfórico 35% por 15s. Provavelmente, o hipoclorito de sódio reagiu

com algumas moléculas de hidroxiapatita da superfície da dentina, assim como com algumas moléculas de carbonato de cálcio e fosfato de cálcio, modificando justamente a superfície em potencial para a adesão, podendo causar diminuição da resistência de união de sistemas adesivos à dentina como visto por Ari et al., (2003) e Ozturk et al., (2004). O conteúdo orgânico por sua vez não foi diferente entre a dentina da câmara pulpar decídua e a permanente, independente dos tratamentos realizados, assim como o hipoclorito de sódio confirmou sua ação desproteinizante tornando o resultado para diferentes grupos homogêneo.

Em síntese, a tese reuniu resultados que contribuem para o estudo do comportamento clínico da dentina decídua de forma abrangente enfatizando aspectos tanto do conteúdo orgânico quanto do conteúdo inorgânico comparativamente à dentina permanente. Assim, vem contribuir com a literatura internacional, por meio dos estudos apresentados nos capítulos 1 e 2, com resultados inéditos sobre a característica da disposição molecular dos elementos químicos componentes da dentina decídua. Além disso, discute a relevância desses achados para as áreas envolvendo Odontologia Restauradora e pontua novas questões para que mais respostas sejam obtidas a partir de futuros estudos.

5. CONCLUSÕES GERAIS

Baseado nos resultados dessa tese pôde-se concluir que:

O conteúdo mineral da dentina decídua apresenta arranjo molecular diferente do conteúdo mineral da dentina permanente, apesar da similaridade da propriedade de dureza entre os dois substratos.

As diferenças do arranjo molecular do conteúdo mineral da dentina decídua em relação à permanente são consistentes visto que o mesmo ocorre na dentina que compõe a câmara pulpar, sendo ambos, decíduos e permanentes, modificados pela ação da solução de hipoclorito de sódio 1%.

REFERÊNCIAS (INTRODUÇÃO E CONSIDERAÇÕES GERAIS)

Akagawa H, Nikaido T, Takada T, Burrow MF, Tagami J. Shear bond strengths to coronal and pulp chamber floor dentin. Am J Dent. 2002; 15(6):383-8.

Angker L, Nockolds C, Swain MV, Kilpatrick N. Quantitative analysis of the mineral content of sound and carious primary dentine using BSE imaging. Arch Oral Biol. 2004 Feb; 49(2):99-107.

Angker L, Swain MV, Kilpatrick N. Micro-mechanical characterization of the properties of primary tooth dentine. J Dent. 2003; 31:261-267.

Araújo FB, Moraes FF & Fossati, ACM. A estrutura da dentina do dente decíduo e sua importância clínica. Rev Bras Odont. 1995; 52(4): 37-43.

Araújo FB. Estudo morfológico, histométrico e histoquímico da polpa de molares decíduos em diferentes fases de reabsorção radicular. [dissertação]. São Paulo: FOUSP; 1982.

Ari H, Yasar E and Belli S. Effects of NaOCl on bond strengths of resin cements to root canal dentin. J End. 2003; 29:248-51.

Ausiello P, De Gee AJ, Rengo S and Davidson CL. Fracture resistance of endodontically-treated premolars adhesively restored. Am J Dent. 1997; 10:237-41.

Borges AFS, Correr GM, Sinhoreti MAC, Consani S, Sobrinho LC, Puppin Rontani RM. Compressive strength recovery by composite onlays in primary teeth. Substrate treatment and luting agent effects. J Dent. 2006; 34:478-484.

Burrow MF, Sano H, Nakajima M, Harada N, Tagami J. Bond strength to crown and root dentin. Am J Dent. 1996 Oct; 9(5):223-9.

Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Effects of storage media on mechanical properties of adhesive systems. Am J Dent. 2004 Apr; 17(2):104-8.

Ciucchi B, Khettabi M, Holz J. The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: a scanning electron microscopic study. Int Endod J. 1989; 22(1): 21-8.

De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005 Feb; 84(2):118-32.

Englert GE, Léon A, Tessele S, Locatelli C. Atomic Force Microscopy – Scannig

Electron Microscopy: comparative evaluation on solid surfaces. Act Mic. 2003 December; 12(1): 107-110.

Gwinnett AJ. Chemically conditioned dentin: a comparison of conventional and environmental scanning electron microscopy findings. Dent Mat. 1994 May;10(3):150-5.

Hiraishi N, Nishiyama N, Ikemura K, Yau JY, King NM, Tagami J, Pashley DH, Tay FR. Water Concentration in Self-etching Primers Affects their Aggressiveness and Bonding Efficacy to Dentin. J Dent Res. 2005 Jul; 84(7):653-8.

Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, Sano H, Van Meerbeek B. Microtensile bond strength of eleven contemporary adhesives to dentin. J Adhes Dent. 2001 Fall; 3(3):237-45.

J.J. Freeman, B. Wopenka, M.J. Silva, J.D. Pateris. Raman Spectroscopic detection of changes in bioapatite in mouse femora as a function of age and in vitro fluoride treatment. Calcif Tissue Int. 2001; 68:156-162.

Johnson, NW, Taylor BB, Berman DS. The response of deciduous dentin to caries studied by correlated light and electron microscopy. Caries Res. 1969; 3: 348-368.

Marshall GW, Yücel N, Balloch M, Kinney JH, Habelitz S, Marshall SJ. Sodium hypochlorite alterations of dentin and dentin collagen. Surf Sc. 2001; 491:444-55.

Misra A, Spencer P, Marangos O, Wang Y, Kate JL. Micromechanical analysis of dentin/adhesive interface by the finite element method. J Bio Mater Res. 2004 Jul 15; 70B(1): 56-65.

Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-Prep on bond strengths of resin cement to endodontic surfaces. J End. 2001; 27: 753-757.

Moutouris G, Silikas N, Eliades G. Effect of sodium hupochlorite treatment on the molecular composition and morphology of human coronal dentin. J Adhes Dent. 2004; 6(3): 175-82.

Nakabaiashi N, Watanabe A, Igarashi K. AFM observation of collapse and expansion of phosphoric acid-demineralized dentin. J Biom Mater Res A. 2004 Mar 1; 68(3):558-65.

Nakabayashi N. Resin reinforced dentin due to infiltration of monomers into the

dentin at the adhesive interface. J Jpn Dent Mat. 1982; 1: 78-81.

Nikaido T, Takano Y, Sasafuchi Y, Burrow MF and Tagami J. Bond strengths to endodontically-treated teeth. Am J Dent. 1999; 12:177-80.

Ozturk B, Ozer F, Belli S. An in vitro comparison of adhesive systems to seal pulp chamber walls. Int End J. 2004; 37(5): 297-306.

Ozturk B, Ozer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. J Endod. 2004; 30(5): 362-5.

Pashley DH, Tao L, Boyd L, King GE, Horner JA. Scanning electron microscopy of the substructure of smear layers in human dentine. Arch Oral Biol. 1988; 33(4):265-70.

Pioch T, Staehle HJ, Duschner H, Garcia-Godoy F. Nanoleakage at the composite-dentin interface: a review. Am J Dent. 2001; 14(4):252-8.

Puppin-Rontani RM & Caldo-Teixeira AS. Effect of Sodium Hipochlorite on the Different Substrates – A SEM Analyses. Act Mic. 2003; 12(1): 169-173.

Reis AF, Arrais CA, Novaes PD, Carvalho RM, De Goes MF, Giannini M. Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression. J Bio Mater Res B Appl Biomater. 2004 Oct 15; 71(1):90-8.

Spears IR. The effect of stiffness on the localization of tensile stress at surface of bonded posterior restorations. Dent Mat. 1998; 14:269-276.

Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers--in situ study. J Dent Res. 2001 Sep 80(9): 1802-1807.

Steinman RR. Physiologic activity of the pulp-dentin complex. Quint Int. 1985 Oct; 16(10):723-6.

Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent. 2002 Winter; 4(4):255-69

Taylor JK, Jeansonne BG, Lemon RR. Coronal leakage: effects of smear layer, obturation technique, and sealer. J End.1997; 23(8): 508-12.

Toba S, Veerapravati W, Shimada Y, Nikaido T, Tagami J. Micro-shear bond strengths of adhesive resins to coronal dentin versus the floor of the pulp chamber.

Am J Dent. 2003; 16 Spec No: 51A-56A.

Tsuda H, Ruben J, Arends J. Raman of human dentin mineral. Eur J oral Sci. 1996; 104: 123-131.

W. Tesh, N. Eidelman, P. Roschger, F. Goldenberg, K. Klaushofer, P. Fratzi. Graded microstructure and mechanical properties of human crown dentin. Calcif Tissue Int. 2001; 69: 147-157.

APÊNDICE

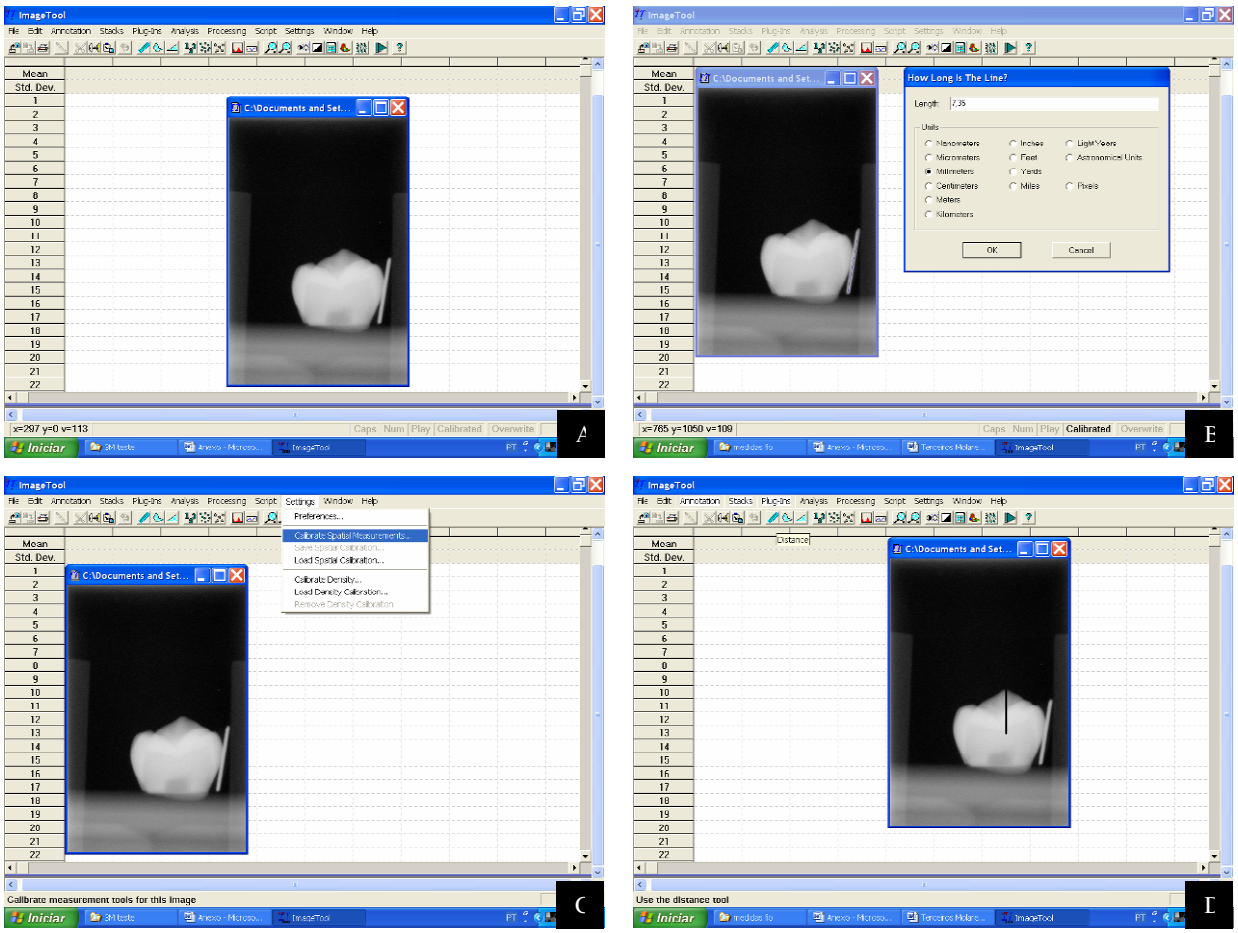


Figura 1. Seqüência da padronização da profundidade de desgaste na dentina utilizando-se radiografias digitais mensuradas no programa Image Tool 3.0. (estudo 1) No caso do exemplo, terceiro molar. **A.** Radiografia do dente com o fio metálico fixo na face proximal. **B.** Calibração do fio metálico cujo valor foi previamente anotado para ser utilizado nesta etapa. **C.** Comando *Calibração de medidas espaciais* acionado para ser realizada a medida convertida automaticamente em milímetros. **D.** Comando distância acionado para ser desenhada uma linha do topo da cúspide mais alta até o terço médio, determinando a profundidade a ser alcançada com o desgaste.

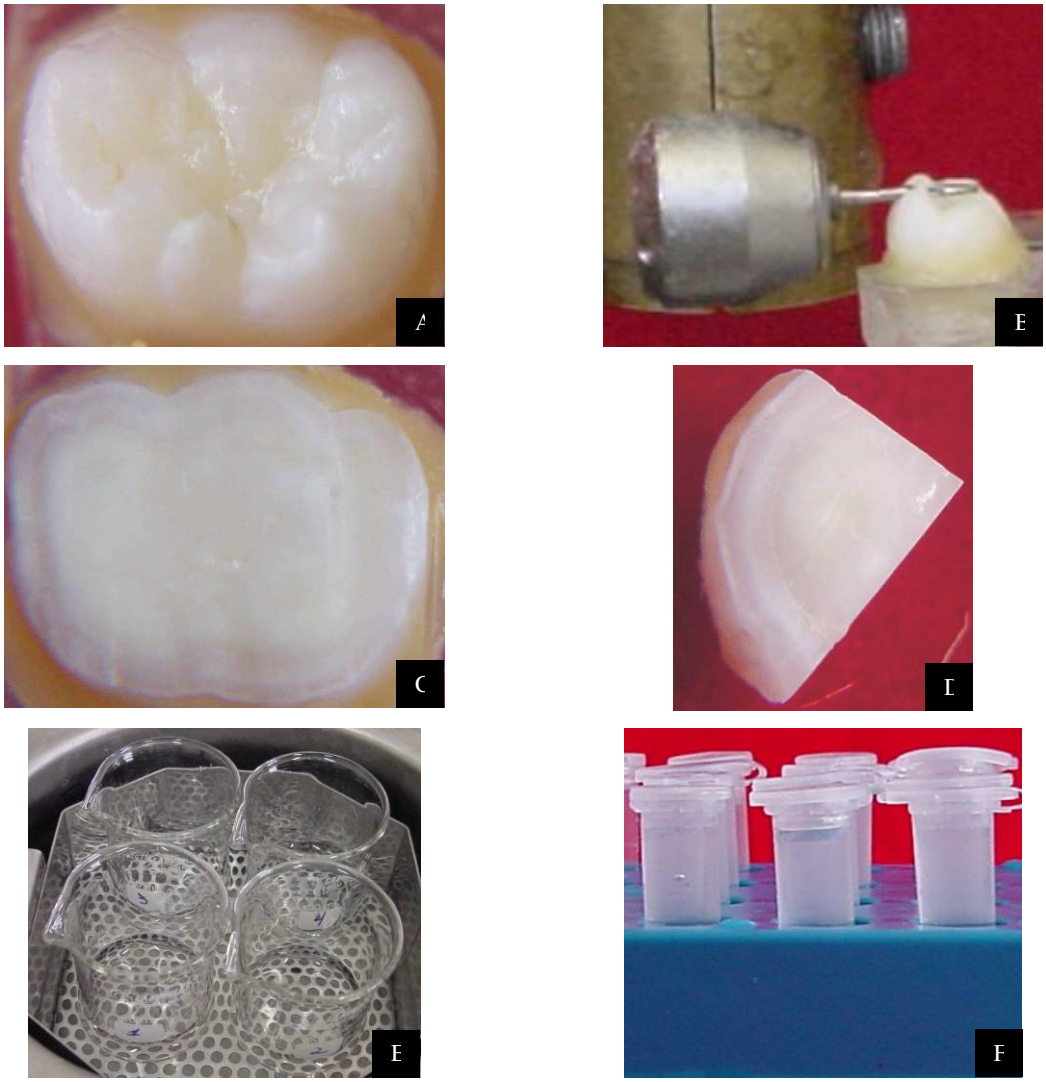


Figura 2. Seqüência de preparo dos espécimes do estudo 1**.** Coroa de um segundo molar inferior decíduo fixa em placa de acrílico por meio de cera pegajosa. **B.** Alta rotação acoplado em máquina padronizadora de preparos com a broca em posição perpendicular à face oclusal da coroa. **C.** Obtenção da dentina média exposta após desgaste **D.** Unidade experimental (1/4 da coroa) obtida após secções mesio-distal e vestíbulo-lingual. **E.** Banho com água deionizada em ultrassom das unidades experimentais agrupadas. **F.** Armazenagem dos espécimes isoladamente em *eppendorfs* após a realização dos tratamentos.

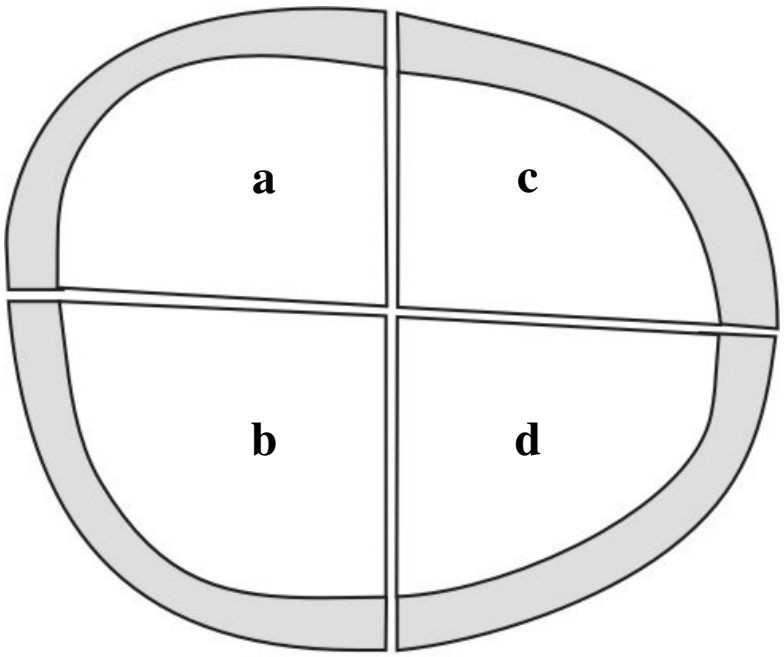


Figura 3. Representação esquemática de um segundo molar inferior decíduo, cujas secções nos sentidos vestíbulo-lingual e mesio-distal proporcionaram a obtenção de 4 unidades experimentais por coroa, divididas entre os grupos de forma dirigida: letras **a** e **d** correspondentes às unidades mésio-vestibular e disto-lingual, respectivamente, foram destinadas ao grupo 1 e as letras **b** e **c**, correspondentes às unidades mésio-lingual e disto-vestibular, respectivamente, foram destinadas ao grupo 2 e assim sucessivamente, até se obter 10 unidades experimentais por grupos, provenientes de 5 molares decíduos e 5 molares permanentes, sendo metade da amostra destinada à análise FT-Raman e a outra metade ao ensaio micromecânico de dureza Knoop.

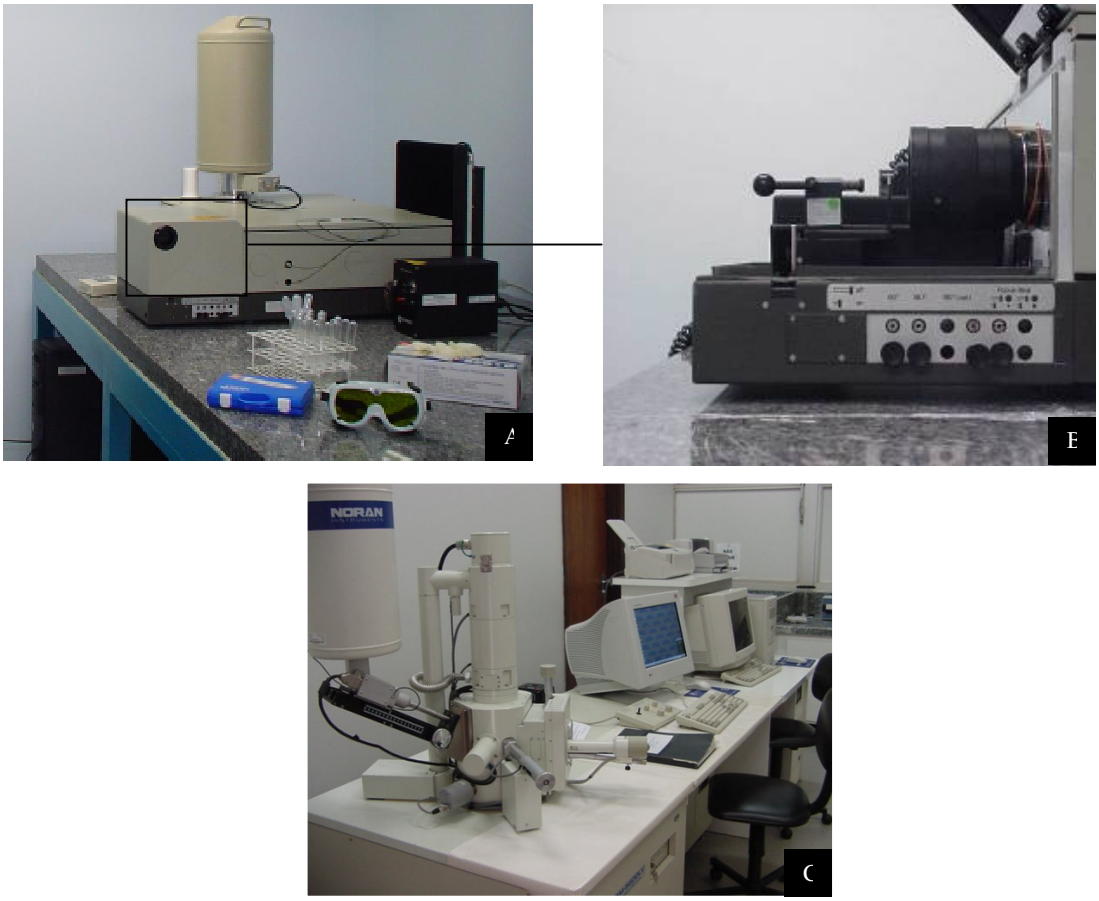


Figura 4. Aparelhos utilizados para realização das análises químicas. **A.** FT-Raman Espectrômetro RFS 100, Bruker. **B.** Geometria de espalhamento e porta amostra do FT-Raman Espectrômetro RFS 100 (Laboratório de Espectroscopia Vibracional – Universidade Vale do Paraíba, São José dos Campos, SP). **C.** Microscópio Eletrônico de Varredura JEOL JSM-5600LV (Tokyo, Japan) utilizado para obtenção de imagens posteriormente medidas pelo Espectrômetro EDS Vantage device (version 1.4 Rev. B, Noran Instruments, Tokyo, Japan - Faculdade de Odontologia de Piracicaba/UNICAMP).

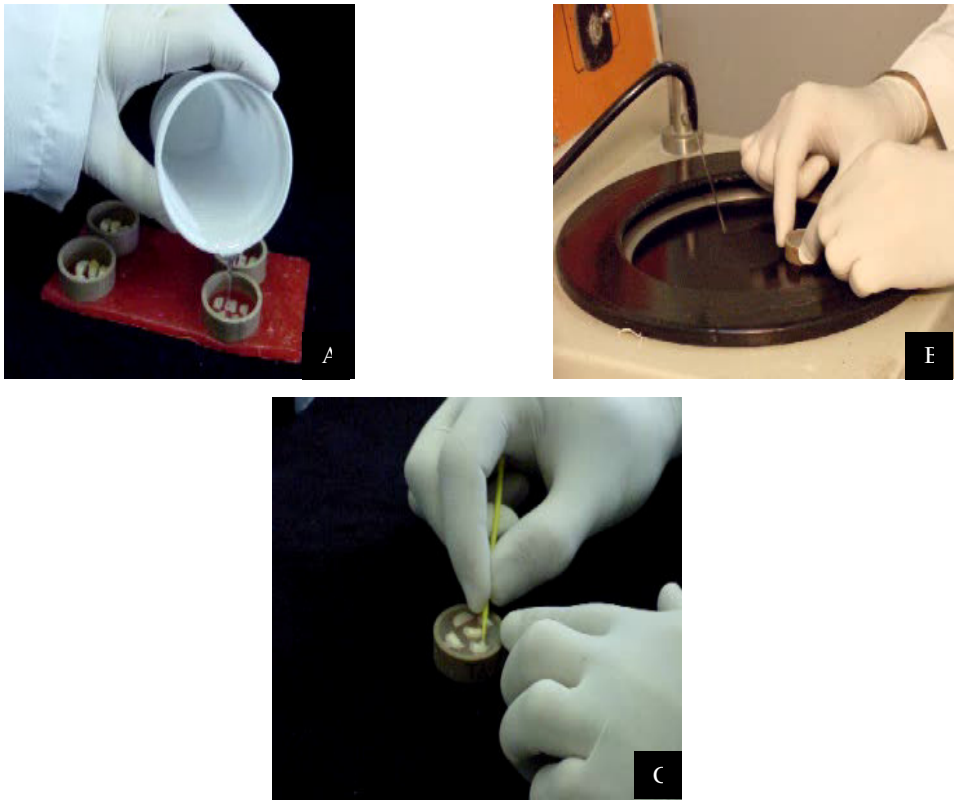


Figura 5. Preparo adicional dos espécimes para o ensaio micromecânico de dureza. **A.** Espécimes incluídos no interior de tubos de PVC, utilizando-se resina de poliestireno (Piraglass Ltda., Piracicaba, S.P., Brasil). **B.** Polimento dos espécimes com lixa de carbeto de silício (Arotec, Cotia, SP) #600 em politriz (Arotec, APL 4, Cotia, SP) sob refrigeração. C. Aplicação do ácido fosfórico 35% (líquido) na superfície dos espécimes dos grupos 2 e 4.

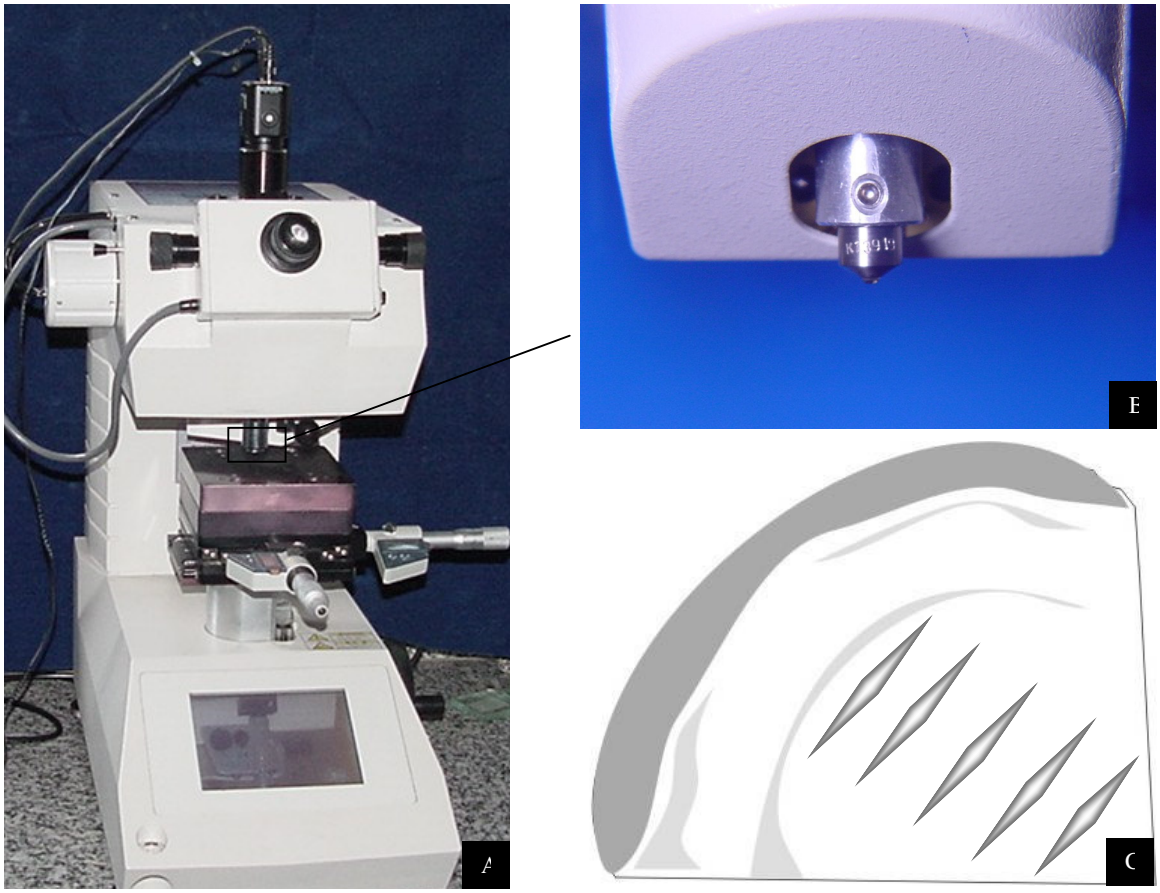


Figura 6. A. Aparelho HMV2 (Shimadzu, Tokyo, Japan), com diamante tipo Knoop **(B)**, utilizado para realização do ensaio micromecânico de dureza. **C.** Representação esquemática das impressões deixadas pelo diamante Knoop na superfície dentinária.

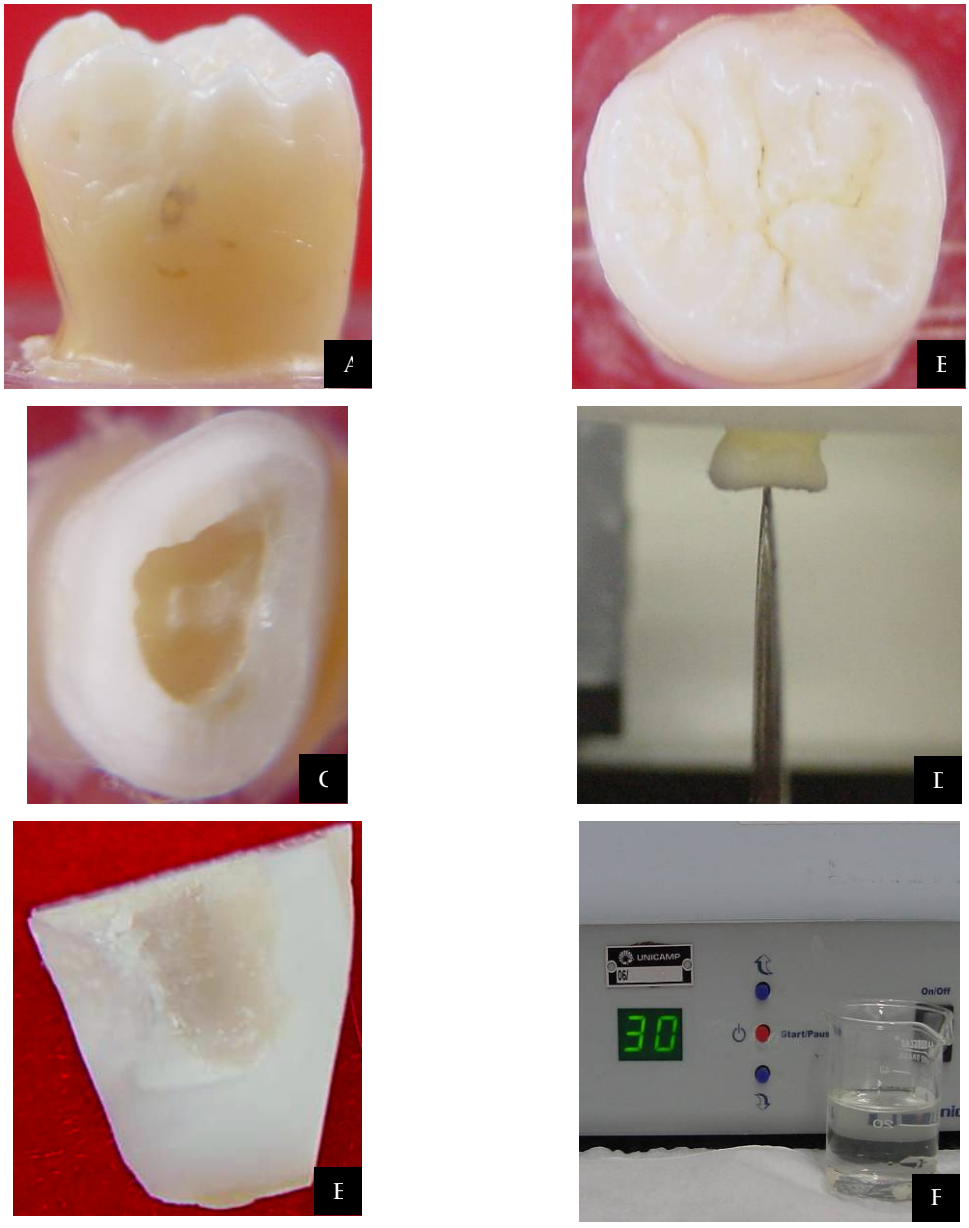


Figura 7. Seqüência do preparo dos espécimes do estudo 2. **A e B.** Vistas vestibular e oclusal de um terceiro molar fixo em placa de acrílico. **C.** Primeiro molar superior decíduo fixo pela face oclusal, dente selecionado como exemplo da seqüência de preparo da amostra. **D.** Secção no sentido mesio-distal da coroa. **E.** Unidade experimental. **F.** Unidades experimentais (n=5) imersas no hipoclorito de sódio 1% (30 min) a frente do ultrassom utilizado.

ANEXO

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Interação molecular da dentina decídua frente a diferentes tratamentos. Análise química, morfológica, micro-mecânica e estudo da interface sistemas de união/dentina**", protocolo nº **083/2005**, dos pesquisadores **ANA FLÁVIA SANCHES BORGES, PATRICIA ALMADA SACRAMENTO e REGINA MARIA PUPPIN RONTANI**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 14/09/2005.

Piracicaba, 29 de setembro de 2006

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Molecular Interaction of deciduous dentin submitted to different treatments. Chemical, morphological, micro-mechanical analysis and adhesive systems/dentin interface study**", register number **083/2005**, of **ANA FLÁVIA SANCHES BORGES, PATRICIA ALMADA SACRAMENTO and REGINA MARIA PUPPIN RONTANI**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 14/09/2005.

Piracicaba, SP, Brazil, September 29 2006

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

Figura 1. Certificado de aprovação do projeto de pesquisa desta tese no Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba/UNICAMP.