UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Influência do material obturador e agentes de limpeza pós-obturação na composição e morfologia da dentina radicular decídua

Dissertação de Mestrado apresentada a Faculdade de Odontologia de Piracicaba, da UNICAMP, para obtenção do título de Mestre em Odontologia, na área de Odontopediatria

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

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Êxodo 35:31-34

O objetivo neste estudo foi avaliar o efeito de diferentes pastas obturadoras e agentes de limpeza sobre a dentina radicular de dentes decíduos, sobre: a estrutura molecular; e a morfologia de superfície da dentina radicular em dentes decíduos. Foram utilizadas 80 raízes de dentes decíduos anteriores extraídos por razões clínicas. As coroas foram seccionadas na junção cemento-esmalte e descartadas; e as raízes seccionadas no sentido longitudinal expondo os condutos radiculares, as quais foram planificadas e sonicadas. As raízes foram fixadas com resina composta para facilitar o manuseio, em seguida os condutos foram instrumentados com limas tipo K (15 a 35) e irrigados a cada troca de lima com hipoclorito de sódio a 0,5% + EndoPTC. Então, os espécimes foram distribuídos em 4 grupos de acordo com as pastas obturadoras (n=20): **Controle-CP** (sem obturação), **Calen® espessada com óxido de zinco-CZ**, **Calcipex II®-CII, Vitapex®-V**. Após a obturação, os espécimes foram armazenamentos por 7 dias. Após esse período, as pastas obturadoras foram removidas e os espécimes foram subdivididos em 4 grupos de acordo com os agentes de limpeza (n=5): **Controle-CL** (Sem limpeza), **Etanol-E**, **Tergenform®-T**, **Ácido fosfórico-AF** 35%. O terço cervical da raiz dos espécimes foram analisados em Espectroscopia Raman Transformada de Fourier (Raman-FT), pico orgânico 2940 cm^{-1} (C-H), e fluorescência de micro RX por dispersão de energia (EDXRF) analise de Ca, P e a razão Ca/P-em % de peso, e em seguida foram desidratados e cobertos com ouro para a análise em Microscopia Eletrônica de Varredura (MEV). Os dados foram submetidos à ANOVA dois critérios e teste Tukey ($p<0.05$). Para as imagens obtidas em MEV foi realizada análise descritiva. Não houve interação entre pastas obturadoras e agentes de limpeza ($p>0,05$). As pastas obturadoras não produziram diferenças significativas no conteúdo orgânico da dentina ($p>0,05$). Entretanto, **CP** apresentou menores valores de Ca, menor razão Ca/P e maiores valores de P quando comparados ao controle. Considerando-se os agentes de limpeza, também não foram observadas diferenças no conteúdo orgânico ($p>0,05$), entretanto, **AF** produziu menores valores de porcentagem em peso de Ca e Ca/P que **T** ($p<0,05$). Para o conteúdo de P, todos os grupos apresentaram resultados similares. Pode-se observar presença de *smear layer* sobre a dentina radicular quando os agentes de limpeza foram utilizados, exceto para o **AF**, o qual produziu túbulos dentinários expostos e superfície dentinária sem resíduos, independente da pasta obturadora utilizada. Pode-se concluir que a composição orgânica da dentina radicular não foi alterada pelas pastas obturadoras. As pastas obturadoras alteraram o conteúdo inorgânico da dentina radicular (Ca, P e Ca/P), porém, os agentes de limpeza não alteraram o conteúdo inorgânico. Quanto à morfologia da superfície da dentina radicular apresentou-se sem presença de smear layer e com túbulos expostos e limpos somente quando o ácido fosfórico utilizado.

Palavras chave: dentina radicular, pastas obturadoras, agentes de limpeza, tratamento endodôntico, dentes decíduos, microscopia eletrônica de varredura, Espectroscopia Raman Transformada de Fourier, Fluorescência de micro RX por dispersão de energia.

The aim of this study was to verify the effect the different filling pastes and cleaning agents in primary dentin root, as: molecular structure and morphologic structure the surface of root dentin in primary teeth. For the study were used 80 anterior primary teeth roots extracted for clinical reasons. The crowns of the teeth were sectioned at cement enamel junction and discarded. The roots were separated longitudinally exposing the root canal, which were ground flat and sonicated. The specimens were fixed using composite resin to facilitate manipulation, and then they were prepared with K-type files size #15-35 and irrigated with of 0.5% sodium hypochlorite solution + EndoPTC cream. Thus, were divided into 4 groups (n=20) according to the filling pastes: **Control** (without filling), **Calen® paste thickened with zinc oxide, Calcipex II**®, **Vitapex**®. Then, the specimens were stored for seven days. After that, filling paste was removed and the specimens were subdivided into 4 cleaning agents groups (n=5): **Control** (without cleaning agents), **Ethanol, Tergenform®**, **Phosphoric acid** (35%). The cervical third of the root canal dentin the specimens were submitted to molecular analyses using FT-Raman spectroscopic (FT-Raman), for analysis of 2940 cm^{-1} (C-H) organic peak, and X-ray fluorescence (EDXRF) for Ca, P elements and Ca/P ratio (in % wt) analysis. The specimens were dried and sputter-coated with gold for morphological analyses in Scanning electron microscopic (SEM). Data were submitted to two-way ANOVA and Tukey tests for comparisons between groups at a significance level set at 5%. For SEM, descriptive statistics was used. There was no interaction between study factors (filling pastes and cleaning agent groups) ($p>.05$). The filling pastes were no observed significant difference in organic content; however, control group showed lower Ca values, Ca/P ratio and higher P values. Considering the cleaning agents, there was no observed difference in organic content ($p>.05$). However, it was observed significant difference between Tergenform and phosphoric acid ($p<.05$), which the phosphoric acid showed values lower Ca and Ca/P ratio. For the P, all the groups showed similar result. It was observed presence of smear layer on dentin when all cleaning agents were used, except for phosphoric acid groups, which presented exposed dentinal tubules and clean surface. It was concluded that the filling pastes and cleaning agents did not provide any changes in organic content the roots primary teeth dentin. The inorganic content in roots primary teeth dentin was change for filling pastes, but it not changes for cleaning agents. Regarding the dentin morphology, the root surface showed no smear layer presence and debris, besides exposed tubules only when phosphoric acid was used.

Keywords: Root dentin, filling pastes, cleaning agents, endodontic treatment, primary teeth, scanning electron microscopy, FT-Raman spectroscopic analysis, X-ray fluorescence analysis.

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Os principais objetivos da Odontopediatria restauradora são reparar ou limitar os danos produzidos pela cárie, proteger e preservar a estrutura dental, restabelecer a função adequada, restaurar a estética e proporcionar a higiene oral adequada. Além de manter a vitalidade pulpar quando possível (American Academy of Pediatric Dentistry – AAPD, 2008/2009).

Apesar do tratamento das lesões de cárie ser iniciado com a paralisação da doença por meio da adequação do meio bucal, procurando controlar e modificar os hábitos alimentares e de higiene do paciente, e posteriormente realizarem-se os procedimentos restauradores para restabelecer as condições funcionais e estéticas da dentição decídua (Nelson-Filho & Silva, 2005; Dhull *et al.,*2011), a prevalência da doença cárie na dentição decídua na população brasileira ainda é altamente significativa. Em levantamento realizado pelo SB-Brasil (2010) observa-se que a média de dentes afetados pela cárie na dentição decídua aos 5 anos de idade é de 2,3 (Ministério da Saúde, 2010). A doença cárie manifesta-se desde a primeira infância sendo considerada a principal responsável pela perda precoce dos dentes decíduos.

Para manutenção e preservação dos dentes decíduos com grandes lesões de cárie e envolvimento pulpar no arco dental o tratamento endodôntico tem sido a escolha, em detrimento da exodontia, pois poderá evitar alterações nas funções mastigatórias e de fonação, além de prevenir o desenvolvimento de hábitos parafuncionais, que favorecem a instalação de maloclusão, e o surgimento de problemas psicológicos, possibilitando que a criança tenha um desenvolvimento saudável (Ounsi *et al*., 2009).

A Odontopediatria busca um material obturador de canais radiculares que seja compatível com os tecidos periapicais, reabsorvível, bactericida e que propicie um bom selamento (Kubota *et al*., 1992; Fuks, 2000). Além disso, para garantir sucesso do tratamento endodôntico, especialmente em dentes decíduos por suas complicações anatômicas, somente a instrumentação do canal não é o suficiente. Faz-se necessário o uso de pastas obturadoras com ação antibacteriana garantindo que não haja reinfecção do sistema de canais radiculares.

Uma ampla variedade de materiais obturadores está disponível no mercado com diversas formulações (Guelmann *et al*., 2004), sendo os mais utilizados à base de óxido de zinco, ou iodofórmio associado ou não ao hidróxido de cálcio (Ranly & Garcia-Godoy, 1991, 2000; American Academy of Pediatric Dentistry – AAPD, 2010/2011); cujos objetivos pleiteados são o controle e a eliminação da infecção para preservação dos dentes permanentes e decíduos (Rifkin, 1980; Fuks, 1999).

A pasta à base de óxido de zinco e eugenol tem sido a utilizada há mais tempo pelos profissionais e pesquisadores (Kubota *et al*., 1992; Cunha *et al*., 2005), entretanto não é considerada histologicamente biocompatível, produzindo reação tecidual inflamatória (Barker & Lockett, 1971; O'Riordan & Coll, 1979; Ranly & Garcia-Godoy, 2000) e reabsorção apical mais lenta (Allen, 1979; Spedding, 1985; Barr *et al*., 1991; Chawla *et al*., 1998; Fuks, 2000). Ainda, há relatos de que pelo fato do material tomar presa (Flaitz *et al*., 1989; Ranly & Garcia-Godoy, 1991; Coll & Sadrian, 1996), poderia prejudicar a formação e irrupção do dente permanente sucessor (Coll & Sadrian, 1996). Além disso, há relatos de citotoxididade causada pelo óxido de zinco eugenol quando utilizado em pastas obturadoras em dentes decíduos, não sendo mais recomendado como material obturador (Geurtsen, 2001).

Dessa forma, os materiais à base de óxido de zinco e eugenol vêm sendo substituídos por materiais à base de iodofórmio e de hidróxido de cálcio (Mortazi & Mesbahi, 2004). Huang *et al.* (2007), afirmaram que o uso de hidróxido de cálcio associado ao iodofórmio, como material obturador em dentes decíduos é a melhor escolha comparado a outros medicamentos.

A pasta Calen® (S.S. White Artigos Dentários Ltda., Rio de Janeiro, Brasil) à base de hidróxido de cálcio associado ao polietilenoglicol 400 (PEG 400), utilizada em dentes permanentes como curativo de demora, tem sido utilizada na pulpectomia de decíduos. O PEG-400, um veículo hidrossolúvel, mantém o hidróxido de cálcio estável por mais tempo na área desejada, prologando a ação, diminuindo a solubilização e aumentando a penetrabilidade na dentina radicular. O hidróxido de cálcio apresenta algumas propriedades físico-químicas desfavoráveis, como permeabilidade tecidual, reabsorvível na região periapical e solúvel no canal radicular (Faria *et al*., 2005; Queiroz *et al*., 2009; Silva *et al*., 2010). Para diminuição da reabsorção do hidróxido de cálcio em dentes decíduos, a pasta Calen® tem sido utilizada espessada com óxido de zinco, em proporções iguais (1:1), idealizando uma reabsorção do material obturador simultaneamente com a reabsorção fisiológica (Silva *et al*., 2010).

Outro material obturador à base de hidróxido de cálcio utilizado na obturação de dentes decíduos é o Vitapex® (Neo Dental International Inc., Federal Way, USA). O Vitapex® é composto por hidróxido de cálcio, iodofórmio e óleo de silicone, tendo como características a ação bacteriostática, bom escoamento, bom contraste radiográfico, alto grau de estabilidade e podendo promover a neorformação óssea. Estudos mostram que o material é de fácil aplicação, radiopaco, não apresenta efeitos tóxicos aos sucessores permanentes e reabsorve um pouco mais rápido do que as raízes dos dentes decíduos (Nurko *et al*., 2000; Ko *et al.,* 2003), entretanto, foi considerada de difícil remoção resultando na aderência a dentina (Hosoya *et al*., 2004). Este aspecto de ser considerado quando da limpeza do conduto na utilização de pinos intra-radiculares, o que poderia diminuir o desempenho adesivo.

Recentemente, foi disponibilizada no mercado odontológico, a pasta Calcipex II® (Nishika, Shimonosski, Japão), um material obturador à base de hidróxido de cálcio, sendo similar à pasta Vitapex®, diferindo apenas no veículo (água destilada) e no contraste (sulfato de bário). E demonstrou ser de fácil remoção do conduto (Hosoya *et al*., 2004; Kim *et al*., 2009).

Segundo estudo de Ozcelik *et al*. (2000) que comparou a tensão superficial de pastas a base de hidróxido de cálcio com diferentes veículos, a baixa tensão superficial aumenta a penetração dos líquidos nas irregularidades do canal e túbulos dentinários. Dessa forma, supõe-se que os diferentes veículos presentes nas pastas obturadoras podem ocasionar diferentes padrões de liberação de substâncias ativas, e, portanto, diferentes graus de penetração na superfície da dentina.

Os veículos PEG-400, água destilada e óleo de silicone presentes nas pastas Calen®, Calcipex ® e Vitapex®, respectivamente, poderiam ter diferentes índices de penetrância da pasta obturadora na dentina radicular e o que poderia deixar resíduos na superfície da dentina, obstruindo os túbulos dentinários.

Como se sabe a restauração do dente tratado endodonticamente é um dos principais responsáveis pelo sucesso da terapia endodôntica em longo prazo. Quando da desobturação parcial do conduto radicular para inserção de pinos intra-radiculares a limpeza da dentina deve ser feita de forma a não deixar resíduos que possam prejudicar a adesão dos materiais restauradores à estrutura dentária, pois a técnica adesiva necessita de substratos limpos e livres de resíduos para que haja a penetração dos monômeros resinosos na dentina intertubular, peritubular e no interior dos túbulos dentinários, formando, assim, uma camada híbrida uniforme com prolongamentos resinosos que garantam a adequada união do material restaurador ao substrato (Nakabayashi *et al.*, 1982; Matos *et al.*, 2008).

Dessa forma, a presença do remanescente de material obturador ou mesmo da "smear layer" produzidos durante o tratamento endodôntico, pode interferir na adesão do agente adesivo utilizado. Sendo assim, para que haja a limpeza e preparo da dentina radicular e o aumento da retenção das restaurações adesivas, faz-se necessário a utilização de agentes de limpeza tais como o ácido fosfórico, o qual promove a remoção da "smear layer", desmineraliza a dentina inter e peritubular e expõe a rede de fibras colágenas, sem que haja a perda das características intrínsicas do conteúdo orgânico da dentina (Borges *et al.*, 2008, 2009).

Outro agente bastante utilizado clínica odontológica é o etanol, um solvente orgânico de óleos (Taylor *et al.*, 1936) o que poderia apresentar uma miscibilidade com as pastas obturadoras e promover um bom desempenho na limpeza da dentina radicular pós desobturação; ou mesmo, lauril dietilenoglicol éter sulfato de sódio (Tergentol® ou Tergenform®) que é um tensoativo aniônico de baixa tensão superficial que penetra nas reentrâncias e ramificações do canal radicular, combinando-se com a “smear layer”, levando-a da superfície dentinária e mantendo-a em suspensão (Matos *et al*., 2008), esta em suspensão pode ser facilmente aspirada/removida do conduto.

A ação tanto das pastas obturadoras, quanto dos agentes de limpeza empregados na terapia endodôntica podem induzir alterações significativas na composição e morfologia da dentina radicular e assim, alterar a adesão de materiais restauradores ou de pinos intra-radiculares a estrutura de dentina radicular tratada.

Como forma de avaliar a composição da dentina radicular a espectroscopia Raman Transformada de Fourier (Raman-FT) tem sido utilizada em pesquisas, tendo como vantagem a possibilidade de se analisar um espécime em condições de umidade, possibilitando a coleta de dados da superfície dentinária desmineralizada, reduzindo o potencial de colapso da rede de colágeno proveniente de dessecação (Tay *et al*., 2002).

Outro método que possibilita a análise da composição da dentina pode ser realizado por meio da fluorescência de micro RX por dispersão de energia (EDXRF). Considerada semi-quantitativa, por meio desta análise podem ser identificados os conteúdos em porcentagem em peso de cálcio (Ca) e fósforo (P), permitindo a obtenção da razão de Ca/P. Esta tecnologia tem sido utilizada para observar a biodegradação de compostos bioativos (Jaakkola *et al*., 2004), avaliar o conteúdo químico de lesões de cárie na dentina (Arnold *et al*., 2003), nanoinfiltração na interface de união resina-dentina (Hashimoto *et al*., 2004), e também para analisar os efeitos da esterilização e laser sobre os componentes da dentina (Soares *et al*., 2011).

Para avaliar basicamente morfologia da superfície de amostras, a Microscopia Eletrônica de Varredura (MEV), tem sido utilizada para observação da penetração de materiais obturadores nos canalículos dentinários (Ruddle, 1992;

Leonard *et al*., 1996; Kouvas *et al*., 1998; Puppin-Rontani & Caldo-Teixeira, 2003; Correr *et al*., 2006; Pascon *et al*., 2007; Borges *et al*., 2008) e identificação de estruturas. Enriquece e acrescenta informações àquelas obtidas pelo método de análise da composição da dentina radicular (Raman-FT e EDXRF).

Sendo assim, esta dissertação[1] tem por objetivo avaliar o efeito de diferentes pastas obturadoras e agentes de limpeza na composição e morfologia da dentina radicular de dentes decíduos.

[1] Esta dissertação foi apresentada no formato alternativo de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

Study of different Post-filling cleaning agents in Primary Teeth filled with different pastes: Molecular and Morphological Analysis of root dentin[2]

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ABSTRACT

Objectives: To verify the effect of different post-filling cleaning agents in primary teeth filled with different filling pastes based on molecular structure (FT-Raman and EDXRF) and morphology (SEM) analyses.

Methods: Eighty anterior primary roots were prepared (irrigation-0.5% NaOCl/EndoPTC plus #35 K-file). They were divided into 4 groups (n=20): **Control-CP** (no filling), **Calen® paste thickened with zinc oxide-CZ, Calcipex II®-CII**, **Vitapex®-V**. After seven days storage (37°C), filling paste was removed and the roots were subdivided into 4 groups (n=5) according to cleaning agents: **Control-C** (no cleaning), **Ethanol-E, Tergenform®-T**, **35% Phosphoric acid-PA**. All specimens were submitted to FT-Raman and EDXRF. Next, they were submitted to SEM. Data from FT-Raman and EDXRF were submitted to two-way ANOVA and Tukey tests ($p<0.05$) and descriptive analysis for SEM.

Results: Regarding filling pastes, there was no significant difference in respect to organic content. However, **CP** provided lower Ca values, Ca/P ratio and higher P values. Cleaning agents provided no difference in organic content compared to control group; however, **T** showed significant lower Ca and Ca/P than **PA**. Regarding P, all groups showed similar results. Smear layer was present on dentin when all cleaning agents were used, except for **PA**, which provided exposed and clean dentinal tubules.

Conclusions: Filling pastes and cleaning agents did not alter the organic content of primary root dentin. The inorganic content in primary dentin roots was changed by filling pastes, but not for cleaning agents. **PA** that provided cleaned and opened tubules.

Keywords: Root dentin, filling pastes, cleaning agents, endodontic treatment, primary teeth, FT-Raman spectroscopic analysis, X-ray fluorescence analysis, Scanning electron microscopy.

1. INTRODUCTION

The endodontic treatment aims to become teeth with irreversible pulpitis into a symptom less until they are lost naturally during the transition from primary to permanent dentition, thus avoiding the extraction and consequently prevent the malocclusion. The adequate endodontic treatment of primary teeth may preserve the arch length, if normal function can be restored, and prevent deleterious oral habits and speech alterations. In addition it maintains normal masticatory function as well as esthetics. The rationale includes the removal of irreversibly inflamed or necrotic radicular pulp tissue by cleaning the root canal system, followed by root canal filling with a material that can be reabsorbed at the same rate as the primary tooth root and be eliminated rapidly if accidentally extruded through the apex.[1,2]

The American Academy of Pediatric Dentistry[2] recommends filling pastes with resorbable material such as nonreinforced zinc/oxide eugenol, iodoform-based paste, or a combination paste of iodoform and calcium hydroxide. After that, the tooth has to be restored with a restorative adhesive material that seals the tooth from microleakage and ensures success.

Different types of filling pastes are used by clinicians and among them calcium hydroxide and iodoform-based pastes. Calen® is a calcium hydroxide-based paste containing polyethylene glycol and zinc oxide thickened;[3] Vitapex®, is composed by iodoform, calcium hydroxide and silicone oil;[4] Calcipex II®, calcium hydroxide composed by barium sulfate and distilled water. In spite of the good effect as biocompatibility, antiseptics and, easily handled and insert in the root canal, they are reabsorbed coincidently with the root reabsortion, antiseptics effect and are easily inserted in the root canal.

Hosoya *et al*.[5] investigated the effect of calcium hydroxide on physical and sealing properties of canal sealers. They showed that Calcipex®, a water-based filling paste, was easy to handle, and also was easily removed. However, since it is very fluid should be great care had to be taken to avoid extrusion via the apical foramen. Whereas, Vitapex®, a silicon oil-based was sticky and adhered to large areas of root canal wall, resulting in significant amounts remaining paste after cleaning root surface. Therefore, it is supposed that Vitapex and Calcipex used as filling pastes have different levels of penetration in the dentin, and have easily to be removed depending on the excipient containing (water – Calcipex, silicon oil-based- Vitapex) .

The quality of the root filling and the restoration sealing are the most important factor for the outcome of endodontic treatment. If they are good it will be improve the endodontic success rate.[6] However, in order to get great restoration longevity the dentin surface has to be free of debris to allow an adequate hybridization of the resin monomers and dentin. The adhesion technique requires the infiltrated monomers polymerized in clean dentin surface, for a successful retention of bonded restoration[7]. This cleaning has to be made on the crown and cervical root surface, since the restorations comprised with composite resin and fiber posts has to remain stable with dentin bonding.

Cleaning agents can be used to get off the dentin of residual filling paste. An anionic detergent solution (Sodium Diethylene glycol Lauryl Ether Sulfate), is used to clean dentin surface due to the reduction of superficial tension of liquids and also leave the debris resulting from root preparation in suspension. However, it has not showed antibacterial activity, since it is a detergent. In addition, it should adequately work on oil removal.[8] Another cleaning agent is the ethanol. It is an oil and polyethylene glycol solvent.[9] Also, is usually founded in primers due to its characteristic volatile. Then, removes fluid excess from the dentinal surface, promoting the follow of the adhesive to the solvent, which lodges deeper and internally in the dentinal tubules, allowing the infiltration of resin monomers into dentin wet, increasing retention of resin.[10,11]

Phosphoric acid used as a etching agent with etch & rinse adhesive dentistry despite it demineralizes the surface substrate in adhesive procedures, it can be considered as a surface cleaning agent that can clean dentin, since it removes the smear layer, demineralizes superficially inter and peritubular dentin and exposes collagen matrix, without changing the intrinsic characteristics of the inorganic content of dentine.[12,13]

Studies showed that the contaminants as handpiece oil,[8,14] saliva and blood[15,16,17] decreased bond strength in dentin. Due to the oily excipient-containing filling paste it would act as contaminants and decrease bonding strength in dentin.

Thus, the aim of this *in vitro* study was to verify the effect the different filling pastes and cleaning agents in primary root dentin cleaning after endodontic procedure. The first tested hypothesis was that dentin surface post-filling root canal is affected by different filling pastes, regarding molecular and surface features. The second tested hypothesis was that dentin surface post-filling root canal is affected by different cleaning agents, regarding molecular and surface features.

2. MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of FOP/UNICAMP (approval No. 120/2009) according to the Brazilian Resolution of the National Commission for Ethics in Research.

2.1. Sample selection

Eighty extracted human anterior primary teeth were cleaned with saline solution to remove the remaining debris and tissue tags. Next, they were frozen stored for no more than 6 months until been used. Selection criteria included those with at least a cervical third of a crown, two thirds of the root length intact, and no previous endodontic therapy.

2.2. Specimen preparation

The crowns of the teeth were sectioned at cement enamel junction (CEJ) using a high-speed diamond disc under water-cooling and discarded. Then, the roots were separated longitudinally using a diamond disc under water-cooling and then cleaved with a knife. Thus, 160 specimens were obtained. These specimens were then examined under stereomicrospe and those that showed cracks or other structural alterations were excluded. Then a total of 80 specimens were selected for this study. The specimens were then ground flat with 320-grit SiC paper, and sonicated for 60 seconds with deionized water.

The specimens were fixed in composite resin and prepared to a working length until the apex using K-type files size #15-35. Between each file using, canal was irrigated with 2 mL of 0.5% NaOCl solution. During preparation it was used EndoPTC cream + 0.5% NaOCl solution. The canal was dried with paper points.

The specimens were equally divided into 4 groups (n=20) according to the filling paste: Control (without filling); **Calen®** paste was thickened by mixing 1.0 g of the paste with 1.0 g of zinc oxide on a glass plate. The resulting material was inserted into the canals using a K-file; **Calcipex II**®; **Vitapex**®. Calcipex II® and Vitapex® were inserted with a pre-packed syringe directly in the canal. Then, specimens were stored in a 100% relative humidity at 37ºC for seven days. Next, all the filling paste visible on the root walls was removed with the aid of curettes and K-files. Subsequently, each group was subdivided into 4 groups (n=5)[12] according to cleaning agents: **control group** (without cleaning agents); file wrapped in cotton soaked in 70% **ethanol** for 10 seconds; file wrapped in cotton soaked in **Tergenform®** for 60 seconds; etched with 35% **phosphoric acid** for 15 seconds and later rinsed for 30 seconds. All materials used and their manufacturers are described on Figure 1. The application times of ethanol and Tergenform® were established in a pilot study. After the specimen's preparation, they were stored in 100% relative humidity at 37ºC until the molecular and morphological analyses.

Also, the filling pastes were used for complementary chemical elemental analysis using X-ray fluorescence (Table 3).

Molecular and morphological analysis of the region analyzed was the cervical third of the root canal dentin.

2.3. Molecular Analysis

The dentin root was analyzed by FT-Raman and EDXRF spectroscopy to evaluate changes in dentin organic and inorganic components, respectively. There was no need for specific sample preparation prior to FT-Raman and EDXRF analyses. Both analytical methods are nondestructive and the data collection was based on laser or x-ray interactions with the sample without contact.

2.3.1*. FT-Raman spectroscopic analysis*

Spectra of the samples were obtained using an FT-Raman Spectrometer (RFS 100=S; Bruker Inc. Karlsruhe, Germany). To excite the spectra, the defocused 1064.1 nm line of a Nd:YAG laser source was used. Maximum incident laser power on the sample surface was about 150 mW and spectrum resolution was 4 cm^{-1}.

The samples were positioned in the sample holder compartment and an IR352 lens collected radiation scattered over 90^{o} on the dentin surface. For each sample, one spectrum was collected at a central point on the dentin root cervical. In order to obtain a good signal to noise ratio, 100 scans were co-added for each spectra. Altogether 80 spectra were obtained.

The changes in the organic dentin components were analyzed by comparing the integrated areas of the Raman peak centered at 2940 cm^{-1}. The integrated areas of the peaks were calculated with Microcal Origin 6.0 software (Microcal Software, Inc., Northampton, MA, USA).

2.3.2. *X-ray fluorescence analysis*

Semiquantitative elemental analyses of calcium (Ca) and phosphorus (P) were carried out with an energy-dispersive micro X-ray fluorescence spectrometer (model mEDX 1300; Shimadzu, Kyoto, Japan) equipped with a rhodium X-ray tube and a Si(Li) detector cooled by liquid nitrogen (N_2) and coupled to a computer system for data processing. The voltage in the tube was set at 15 kV, with automatic adjustment of the current. Three spectra from each specimen were collected after the treatments. The measurements were performed with a count rate of 100 sec per point. The equipment calibration and chemical balance were performed as previously reported.[18]

2.4. Morphological Analysis

2.4.1. ***Scanning electron microscopic analysis***

The specimens from all groups were dried and mounted on a holder using double-sided adhesive carbon tape. The samples were sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein) and examined with a scanning electron microscope (JEOL JSM 5600 LV, Tokyo, Japan) operating at 1000x magnifications.

2.5. Statistical Analysis

Statistical analysis was performed with two-way ANOVA followed by Tukey's test using Assistat 7.6 beta (Campina Grande, PB, Brazil), and significance level was set at 5%.

3. RESULTS

According to two-way ANOVA statistical analysis, there was no interaction between filling paste and cleaning agent studied factors ($p >= .05$). Considering the filling pastes on FT-Raman (2940 cm^{-1} band) analysis, no significant difference was found between filling pastes ($p >= 0.05$). Filling pastes did not produce any change on organic content of the dentin. However, when Ca, P and Ca/P ratio were determined by EDXRF analysis, it could be observed significant differences between control and filling paste groups. Ca content and Ca/P ratio of control group was significantly lower than those of filling paste groups with no difference between them ($p >= 0.05$). For P content, the opposite was verified, control group showed higher values compared to those using filling paste groups ($p<0.05$) (Table 1).

Regarding cleaning agents, for all groups there was no significant difference between them ($p>0.05$), concerning FT-Raman analysis. However, Tergenform® showed higher values for Ca content and Ca / P ratio than phosphoric acid .All groups were similar for P content, concerning EDXRF analysis (Table 2).

The chemical elements of the filling pastes identified and quantified by EDXRF are shown on Figure 2 and Table 3, respectively. Table 3 shows that all filling paste contained calcium in their composition. For Calen® + Zinc Oxide filling paste contained zinc in higher proportion than calcium. The Calcipex II® filling pastes contained barium, titanium and sulfur, but calcium in higher percentage. The Vitapex® filling paste contained iodine and silicon in higher proportion than calcium.

Morphological analysis of root canal dentin surfaces of primary teeth using SEM showed retained filling paste amount and smear layer upon dentin surface for all cleaning agent groups, except when phosphoric acid was used. It completely removed the filling pastes on dentin surface (Figure 3). For control group (no filling pastes) and no cleaned up or cleaned up with Ethanol or Tergenform® similar characteristics of dentin surface was found: dentin tubules and dentin surface covered by dense smear layer. While cleaned up with phosphoric acid dentin surface of root canal filled up with different filling pastes showed intertubular dentin with a corroded aspect, visible dentin tubules and peritubular dentin demineralized with wide tubule entrance. The other groups, otherwise, showed some areas with lower corroded intertubular dentin, however, they were all cleaned up from filling pastes. Apparently, specimens filled up with Vitapex® showed remaining residues even after use phosphoric acid. Overall, all filling pastes, Ethanol and Tergenform®, used in this study, did not showed any ability to remove smear layer and showed a dirty surface with filling pastes remaining residues on dentin surface.

Figure 1. Details of manufacturer.

	Manufacturer / Batch Lote	Contents
K-type file	Dentsply Maillefer, BallaguesSwitzerland /#8313220	--
EndoPTC cream	Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil /#83510	urea peroxide, polysorbate 80, polyethylene glycol
0.5%Sodium Hypochlorite	RioLab, Brazil /#CH95436	sodium hypochlorite solution (10-12%)
Calen®	S.S.White Artigos Dentários Ltda., Rio de Janeiro, Brazil /#0061010	calcium hydroxide (2.5 g), zinc oxide (0.5 g), colophony (0.05 g), polyethylene glycol 400 (1.75 mL)
Zinc Oxide	Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil /#32610	Zinc Oxide (100%)
Calcipex II®	Nishika, Shimonoseki, Japan /#Y5N	calcium hydroxide (24%), barium sulfate(24%), distilled water (52%)
Vitapex®	Neo-Dental, Tokyo, Japan /#ZD01	calcium hydroxide (30.3%), iodoform (40.4%), silicone oil (22.4%), excipients (6,9%)
70% Ethanol	Carlos Erba Reagents, Italy /#9F 216019	Ethanol absolute
Tergenform®	F&A Laboratório Farmacêutico Ltda., São Paulo, SP,Brazil /#8172	Sodium Diethylene glycol Lauryl Ether Sulfate - manufacturer' information
Phosphoric Acid	3M ESPE, St. Paul, MN,EUA /#N187625	Phosphoric Acid (35%)

Table 1. Mean and standard deviations of Calcium (wt %), Phosphorus (wt %) and Ca/P ratio concerning X-ray fluorescence analysis, and FT-Raman spectroscopic analysis for peak area (2940 cm^{-1}) in primary root dentin for the filling pastes.

Pastes	EDXRF			FT- Raman 2940 cm^{-1}
	Ca	P	Ca/P	
Control	21.57±0.82 b	12.15±0.73 a	1.77±0.04 b	1.08±0.14 a
Calen® + Zinc Oxide	23.71±1.08 a	10.77±0.76 b	2.22±0.12 a	1.06±0.13 a
Calcipex II®	23.51±1.04 a	9.98±0.81 b	2.41±0.27 a	1.04±0.10 a
Vitapex®	24.15±1.63 a	10.80±0.44 b	2.25±0.16 a	1.04±0.09 a

A lowercase letter following a value indicates significant differences between groups.

Table 2. Mean and standard deviations of Calcium (wt %), Phosphorus (wt %) and Ca/P ratio concerning X-ray fluorescence analysis and FT-Raman spectroscopic analysis for peak area (2940 cm^{-1}) in primary root dentin for the cleaning agents.

Cleaning Agents	EDXRF			FT- Raman 2940 cm^{-1}
	Ca	P	Ca/P	
Control	22.97±2.10 ab	10.65±1.18 a	2.20±0.30 ab	1.05±0.06 a
Ethanol	23.58 ±1.05 ab	10.92±1.20 a	2.20±0.31 ab	0.94±0.05 a
Tergenform®	24.06±1.13 a	10.87±1.45 a	2.28±0.40 a	1.18±0.06 a
Phosphoric Acid	22.33±1.33 b	11.26±1.20 a	1.99±0.10 b	1.04±0.08 a

A lowercase letter following a value indicates significant differences between groups.

Table 3. Results obtained by X-ray fluorescence analysis show the chemical elements Silicon (Si), Sulfur (S), Calcium (Ca), Titanium (Ti), Zinc (Zn), Iodine (I), Barium (Ba) identified in filling pastes in percentage.

Elements	Calen® + Zinc Oxide (%)	Calcipex II® (%)	Vitapex® (%)
Si	-	-	42.29
S	-	8.14	-
Ca	26.79	40.48	27.91
Ti	-	12.46	-
Zn	73.21	-	-
I	-	-	29.80
Ba	-	38.92	-

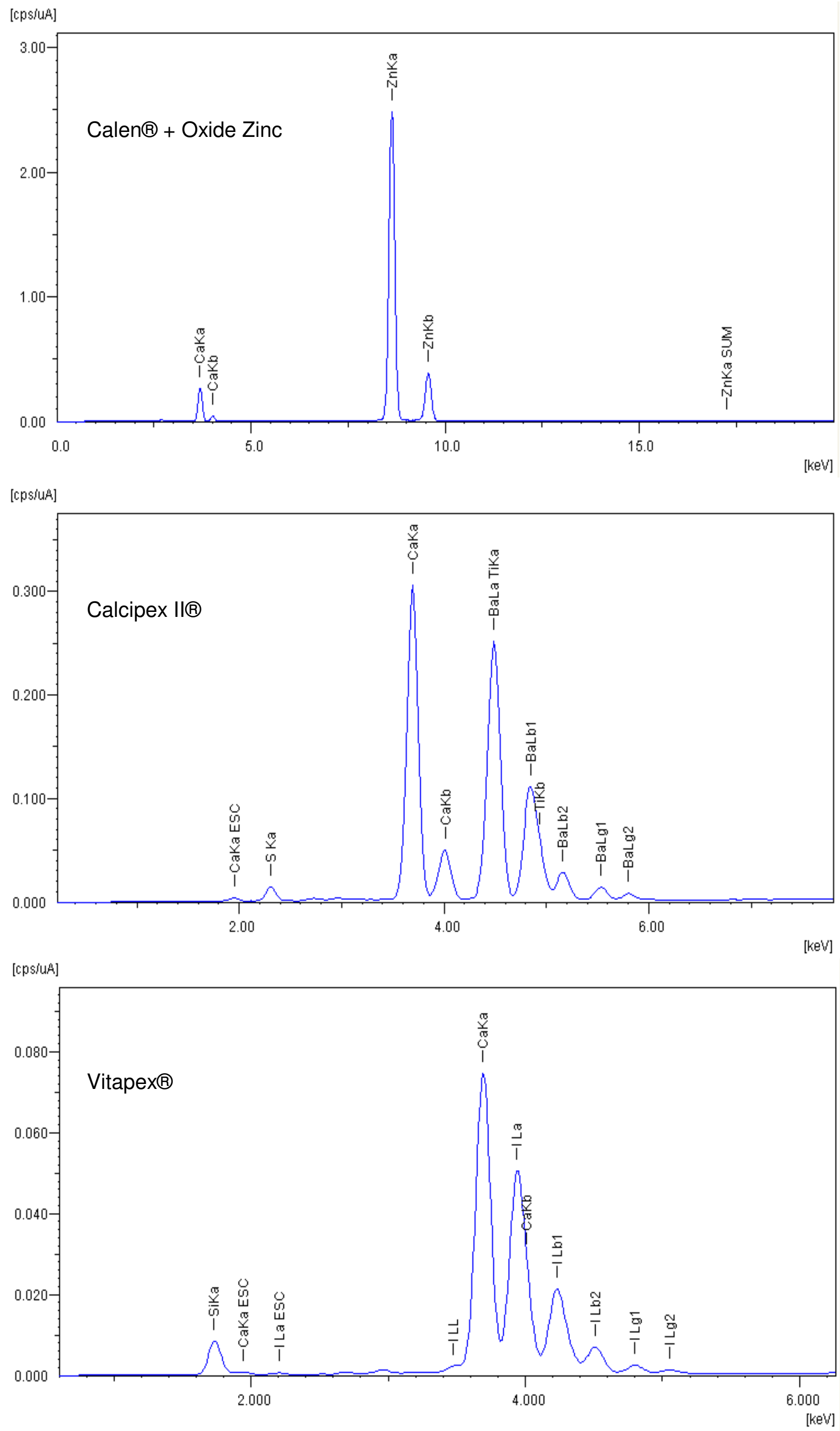

Figure 2. Chemical elements spectra (Silicon (Si), Sulfur (S), Calcium (Ca), Titanium (Ti), Zinc (Zn), Iodine (I) and Barium (Ba) showed by X-ray fluorescence analysis line-scan in the filling pastes groups.

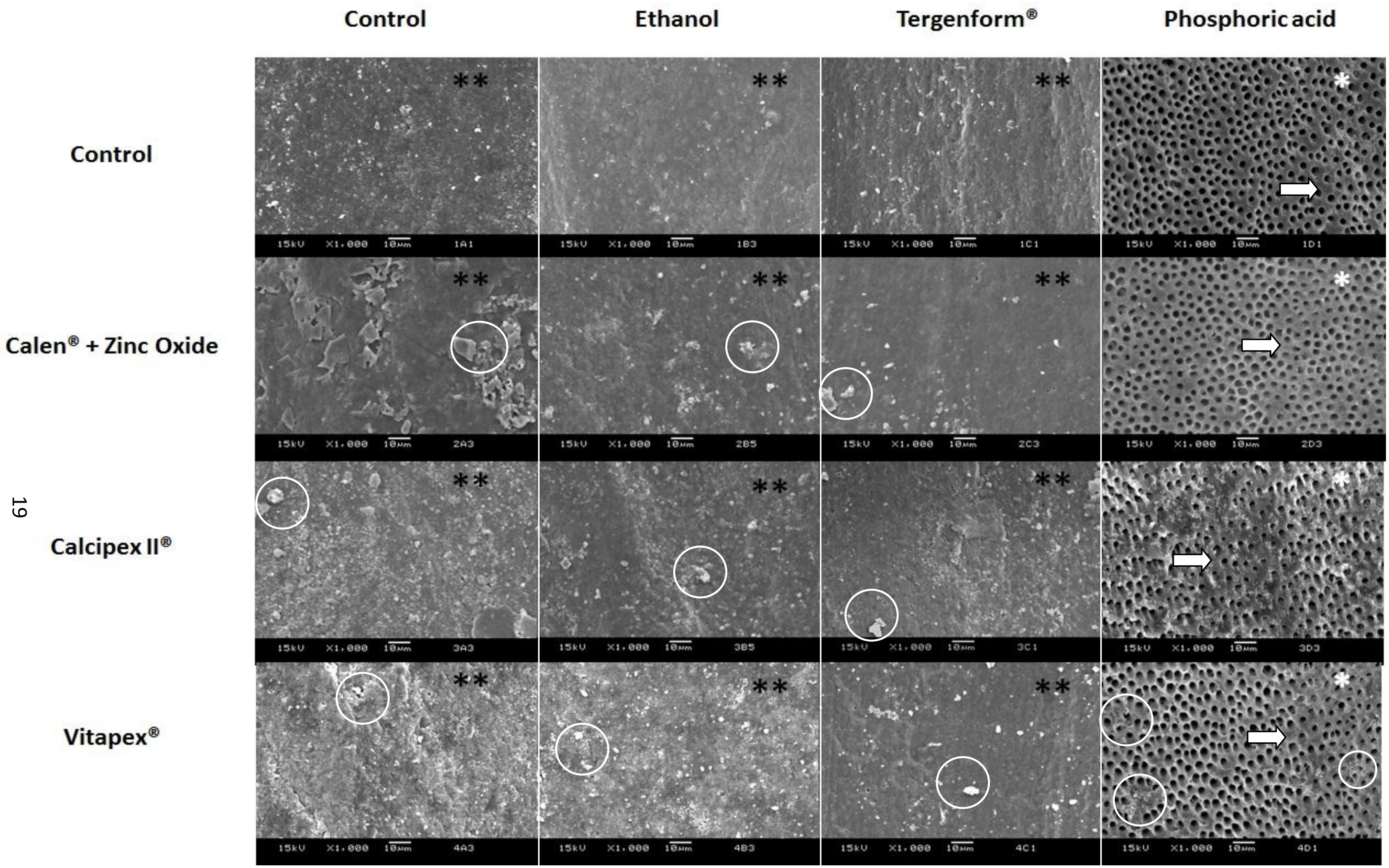

Figure 3. Representative SEM images of root dentin surfaces of primary teeth, regarding the pastes and cleaning agents (original magnifications: 1000x). SEM observations revealed the presence of a heavy smear layer (**) in all groups. For the phosphoric acid group, no smear layer was present, and all tubules were cleaned and opened (*). White circles show particles the filling pastes remaining on dentin surface. White arrows point out no demineralised intertubular dentin area. White circles mean filling paste residues.

4. DISCUSSION

The first tested hypothesis in this study that there were differences between filling pastes considering the molecular features (inorganic and organic content) of primary root dentin was partially accepted. There were significant differences in the inorganic content regarding Ca, P and Ca/P for analysis of EDXRF when compared control and filling pastes. For Ca and Ca/P, the values in weight % were higher for filling paste groups compared to control one, and for P the values were lower than control group. Also, between the filling pastes there were no significant differences. However, there were no significant differences in the organic content of dentin when FT-Raman analysis was done both filling pastes and cleaning agents.

The increased Ca values it could be since all filling pastes tested were calcium-based (Table 1) and they remained on dentin surface over the with smear layer. However, based on the microanalysis of chemical containing pastes, it could be noticed that the Ca amount on filling pastes it is not essential to increase the Ca on dentin, since Calcipex II® presented the highest Ca (Table 3) amount and it not provided a highest Ca weight % (Table 1) available on dentin as compared with the other filling pastes. By the other hand, P content was higher on control group than the filling pastes. It could be observed that the filling pastes that remained on dentin surface over the smear layer, probably blocked or stopped phosphate analysis. Similar results were described for Rythén et al.[19] in exfoliated primary teeth dentin analysis using X-ray microanalyses which showed that Ca values were higher when than P values were lower.

Regarding the cleaning agents the second tested hypothesis was partially accepted, since there was no significant difference between filling pastes and control group related to organic content, but there was significant difference related to inorganic content. (Table 2) There were no significant differences in the organic content when the cleaning agents were used. The 2940-2942 cm^{-1} band (C-H stretching) was used to semi-quantify the relative organic changes, since it is sharper and stronger than those amides bands.[12,13,20,21] This study showed that as such the filling pastes as cleaning solution did not provide change in collagen. This is clinically important, because dentin is composed of 20% in weight[22,23] or 30% in volume[23] hydrated organic matrix, most of which consists of collagen. It constitutes ~90% of the organic matrix.[23] This notice can be good for bonding of current one-bottle adhesive systems that acid-etch the dentin relies on resin-infiltration and encapsulation of collagen fibrils in the wet demineralized dentin to form the hybrid layer or resin-dentin interdiffusion zone. Ideally, this layer/zone is a structurally integrated resin-collagen biopolymer hybrid that provides a continuous and durable link between the bulk adhesive and dentin substrate.[11] Similar results were found regarding the organic content of primary dentin when acid etch was used.[12,21]

Primary root dentin cleaned up with Tergenform® showed higher content of Ca and Ca/P ratio than phosphoric acid and did not show significant differences between the other groups. Tergenform® to be a detergent with alkaline pH showed no dentin demineralization or not remove the filling pastes even after cleaning. Already, the phosphoric acid demineralized dentin surface with loss of Ca. Although there was difference between them, they were similar to control group. Be the phosphoric acid similar the control group, which probably explains it, that is higher calcium present in filling pastes would buffer phosphoric acid, and this effect is associated with pH increasing, and the demineralization rate decreased in those groups,[24] without time interference.[25] Other explanation or effect additional, would be the high carbonate content in primary dentin that is inversely proportional to calcium content,[26] that probably contributed or added to the buffering effect. In addition, regarding the buffering effect, while acid etching removes up to 30% of the calcium phosphates, it dissolves 75% of the carbonates in the same dentin particles.[27] Therefore the calcium phosphate was loss affected, while the carbonate was removed, showing that Ca% was similar in etched phosphoric acid and control groups. Studies from Borges *et al*. (2007, 2008) [12,21] showed that there are no difference on inorganic content of dentin pulp chamber when phosphoric acid is or not used to etch dentin.

Representative SEM images of root dentin surfaces of primary teeth showed tubules clean and open when all filling paste groups were used and were etched by phosphoric acid. Borges *et al.*[12] also showed that primary dentin etched by phosphoric acid enhanced tubules open and enlarge.

Representative SEM images of root dentin surfaces of primary teeth for the group Vitapex® filling paste and phosphoric acid showed areas with remaining paste. Hosoya *et al.* (2004),[5] described that Vitapex® was sticky and adhered to large areas of root canal wall, resulting in significant amounts of remaining paste, even after cleaning procedures. Nevertheless, other cleaning agents studied showed tubules recovered by a heavy smear layer and filling pastes debris. To our knowledge, there have been no reports that verified the molecular and morphological features of root dentin of primary teeth when filling pastes and cleaning agents were used.

Results from this study provided important information concerning the effect of filling root canal pastes used on pediatric dentistry and the possible cleaning agents used to remove these filling pastes in order to enhance the restorative procedure. In fact, it could be observed that all filling pastes studied could remain on dentin surface of root canal and phosphoric acid use is possible to clean them providing an adequate surface for bonding, as commonly known that effective the adhesion technique requires the infiltrated monomers polymerized in dentin clean.[7] Showing that it is not possible after the use of filling pastes the adhesion without etching acid step, can be unviable the use of one step adhesive.

5. CONCLUSION

This *in vitro* study concluded that, filling pastes and cleaning agents studied did not alter the organic content of primary root dentin. The inorganic content in roots primary teeth dentin was change for filling pastes, but it not changes for cleaning agents. Morphological features of primary root dentin were affected by phosphoric acid group treatment, showing cleaned and opened dentin tubules.

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Baseando-se nos resultados obtidos, pode-se concluir que:

1. As pastas obturadoras e os agentes de limpeza não alteraram o conteúdo orgânico da dentina radicular de dentes decíduos.
2. O conteúdo inorgânico da dentina foi alterado pelo uso das pastas obturadoras, mas não foi alterado pelos agentes de limpeza. Porém, o uso do ácido fosfórico produziu diminuição na porcentagem em peso de Ca e na razão Ca/P quando comparado ao Tergenform.
3. Quanto ao aspecto morfológico, a superfície da dentina radicular apresentou-se sem presença de *smear layer* com túbulos expostos e limpos somente quando o ácido fosfórico foi utilizado.

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* De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors-Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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Tabela. Média e desvio padrão de todos os grupos.

Grupos	Ca	P	Ca/P	FT-Raman
controle	21,18±2,19	12,07±0,92	1,75±0,05	1,10±0,28
controle - etanol	22,09±1,84	12,51±0,70	1,76±0,06	0,95±0,24
controle - Tergenform	22,39±2,18	12,86±1,03	1,74±0,04	1,26±0,16
controle - ácido fosfórico	20,61±3,63	11,17±1,64	1,84±0,07	0,99±0,32
Óxido de Zinco - controle	22,18±1,88	9,77±1,30	2,28±0,22	1,07±0,39
Óxido de Zinco - etanol	24,58±2,74	11,11±1,67	2,24±0,35	0,88±0,16
Óxido de Zinco - Tergenform	24,33±1,80	10,66±1,32	2,32±0,42	1,13±0,55
Óxido de Zinco - ácido fosfórico	23,75±2,19	11,56±0,91	2,06±0,13	1,17±0,28
Calcipex - controle	22,50±1,07	9,59±1,79	2,40±0,40	0,96±0,31
Calcipex - etanol	23,91±1,19	9,77±1,21	2,48±0,36	0,99±,013
Calcipex - Tergenform	24,79±0,81	9,39±1,68	2,71±0,51	1,18±0,24
Calcipex - ácido fosfórico	22,83±1,35	11,17±0,72	2,05±0,13	1,01±0,04
Vitapex - controle	26,00±1,41	11,18±1,35	2,35±0,28	1,07±0,28
Vitapex - etanol	23,74±1,22	10,28±1,23	2,33±0,21	0,94±0,23
Vitapex - Tergenform	24,72±1,88	10,59±0,74	2,34±0,14	1,16±0,24
Vitapex - ácido fosfórico	22,14±1,01	11,15±1,25	2,01±0,27	1,01±0,29

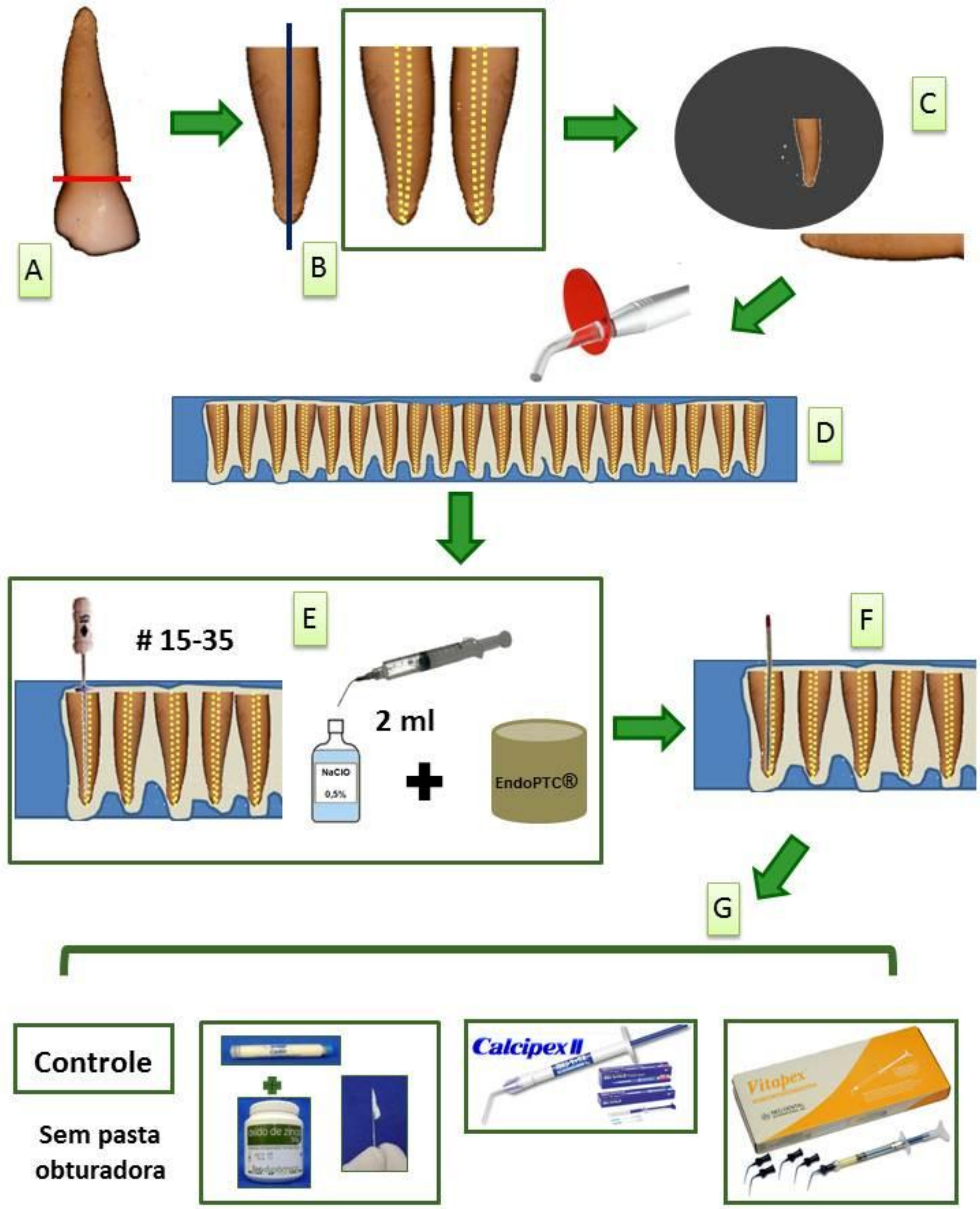

Figura 1. Material e métodos. (**A**) as coroas foram seccionadas na junção cemento-esmalte e descartadas; (**B**) as raízes seccionadas no sentido longitudinal expondo os condutos radiculares; (**C**) planificadas e sonicadas; (**D**) fixadas com resina composta para facilitar o manuseio; (**E**) os condutos instrumentados com limas tipo K (15 a 35) e irrigados a cada troca de lima com hipoclorito de sódio a 0,5% + EndoPTC; (**F**) condutos secos com papel absorvível; (**G**) os espécimes distribuídos em 4 grupos: Controle (sem pasta obturadora), Calen® espessada com óxido de zinco, Calcipex II®, Vitapex®.

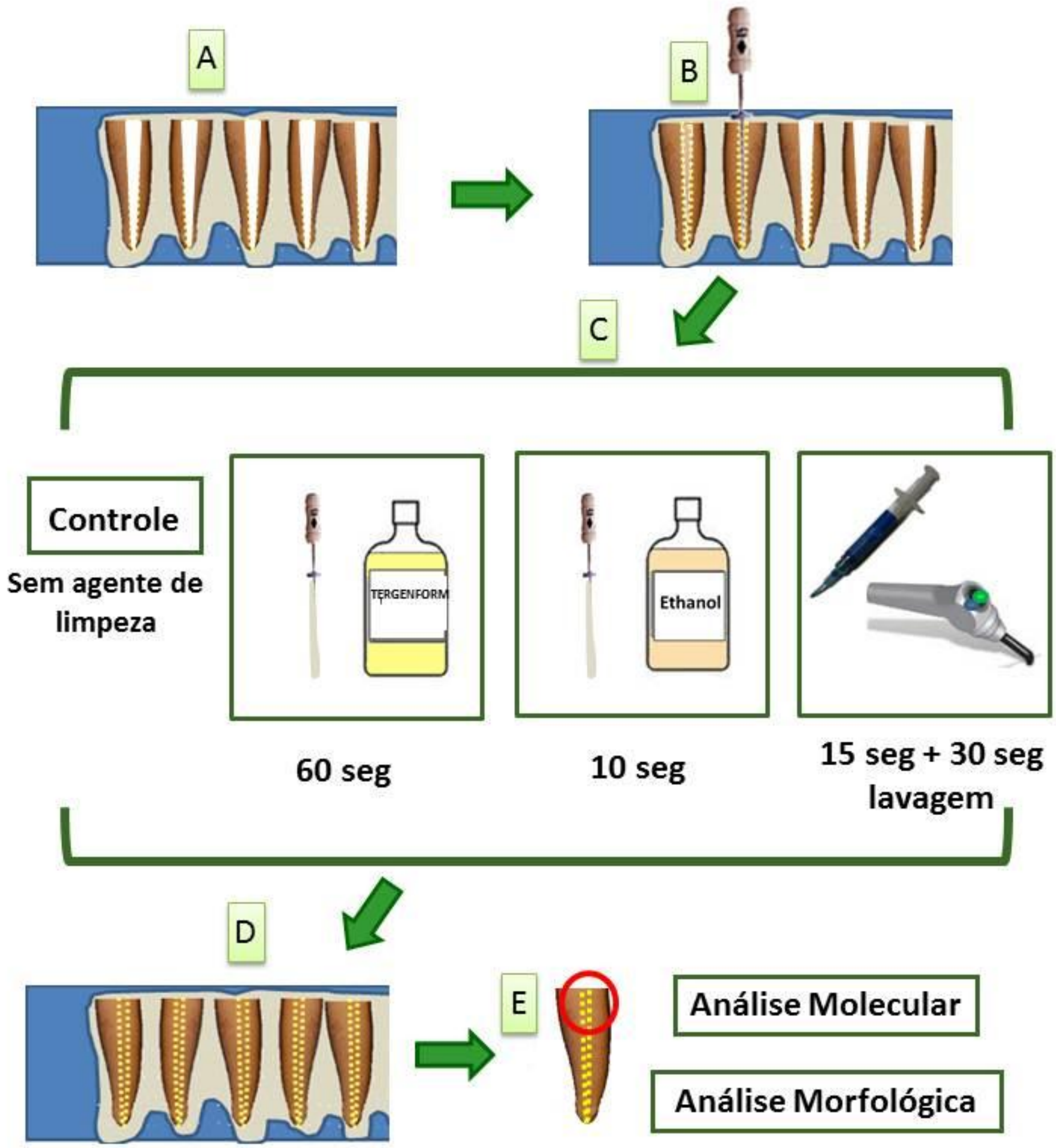

Figura 2. Material e métodos. (**A**) após a obturação, os espécimes foram armazenamentos por 7 dias; (**B**) remoção das pastas obturadoras com auxílio de lima; (**C**) espécimes subdivididos em 4 grupos: Controle (Sem agente de limpeza), Tergenform® por 60 segundos, Etanol por 10 segundos, Ácido fosfórico 35% por 15 segundos e lavagem com água com seringa tríplice por 30 segundos; (**D**) espécimes armazenados até o momento das análises; (**E**) terço cervical da raiz dos espécimes analisados.

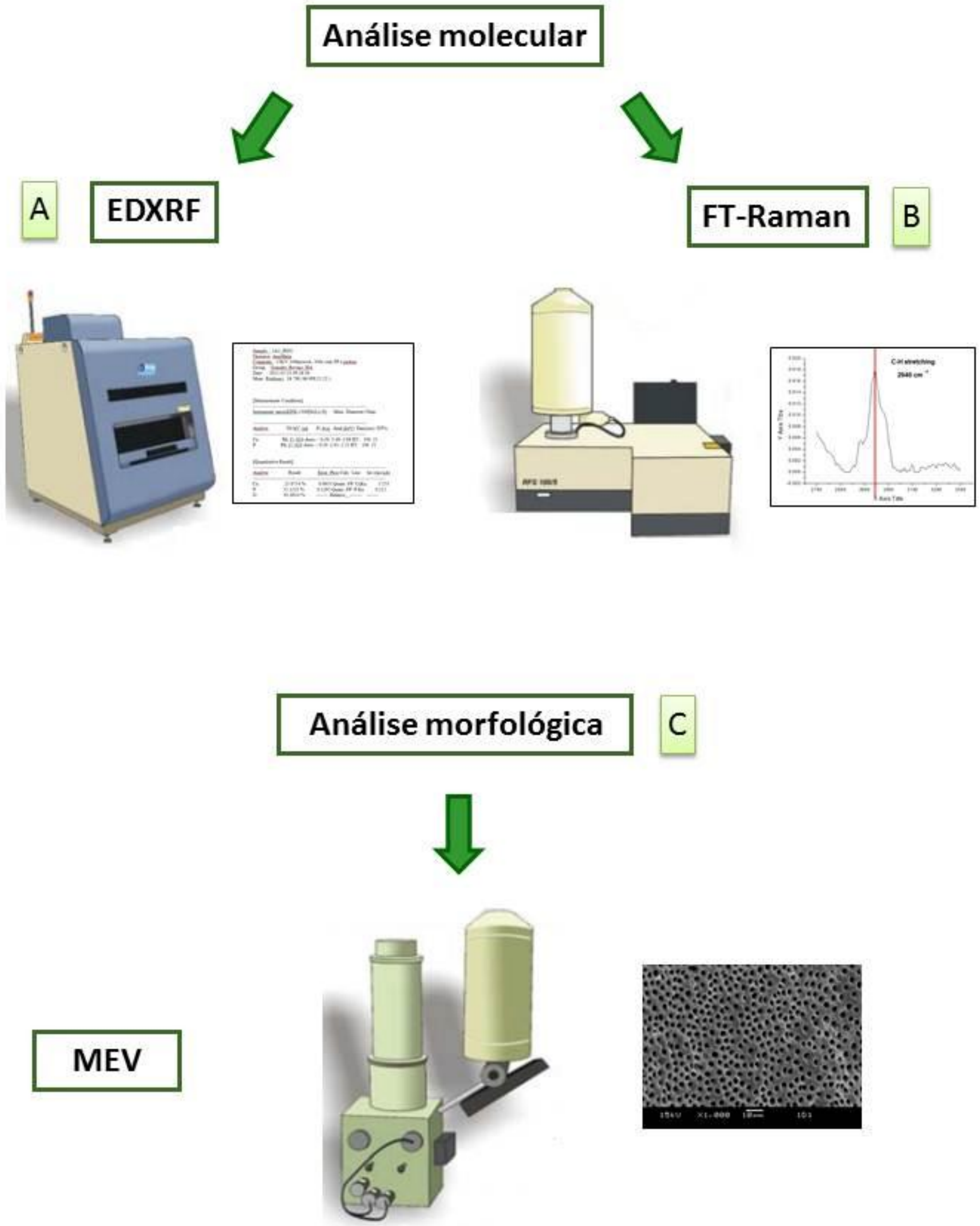

Figura 3. Análise molecular e morfológica. (**A**) Fluorescência de Micro RX por Dispersão de Energia (EDXRF) analise de Ca, P e a razão Ca/P em % de peso; (**B**) Espectroscopia Raman Transformada de Fourier (Raman-FT), pico orgânico 2940 cm^{-1} (C-H); (**C**) Microscopia Eletrônica de Varredura (MEV), análise das imagens obtidas.

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 **"Estudo de diferentes agentes químicos na limpeza pós-obturação com diferentes excipientes em dentes decíduos: Análises molecular, morfológica e micro-mecânica da dentina radicular"**, protocolo nº 120/2009, dos pesquisadores Regina Maria Puppin Rontani e Vanessa Benetello, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 17/09/2009.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Study of the different post-filling cleaning agents in primary teeth filled with different excipient pastes: Molecular, morphological and micro-mechanical analysis of root dentin"**, register number 120/2009, of Regina Maria Puppin Rontani and Vanessa Benetello, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 09/17/2009.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
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.

-----Mensagem Original-----
From: Journal of Dentistry
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To: rmpuppin@fop.unicamp.br
Subject: Submission Confirmation for Journal of Dentistry

Dear Dr Puppin-Rontani,

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