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Dissertação

Atividade antibacteriana e propriedades físico-químicas de um novo material obturador endodôntico resinoso contendo óleos de Butiá ou Copaíba

Cristiane Marcant Reiznautt

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“Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana.”

(CARL JUNG)

Notas Preliminares

A presente dissertação foi redigida segundo o Manual de Normas para trabalhos acadêmicos da Universidade Federal de Pelotas, adotando o Nível de Descrição em Capítulos não convencionais. Disponível no endereço eletrônico: http://sisbi.ufpel.edu.br/arquivos/PDF/Manual_Normas_UFPel_trabalhos_acad%C3%AAmicos.pdf.

O projeto de pesquisa contido nesta Dissertação é apresentado em sua forma final após qualificação realizada em 17 de fevereiro de 2016 e aprovado pela Banca Examinadora composta pelos Professores Doutores Renata Morgental, Adriana Silva, Gabriela Basso (suplente).

Resumo

REIZNAUTT, Cristiane Marcant. **Atividade antibacteriana e propriedades físico-químicas de um novo material obturador endodôntico resinoso contendo óleos naturais de Butiá ou Copaíba.** 2017. 56f. Dissertação (Mestrado em Odontologia) - Programa de Pós Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas, 2017.

O objetivo do presente estudo foi o desenvolvimento de um cimento endodôntico resinoso dual contendo fitoterápicos que apresentem efeito antibacteriano e não sejam citotóxicos, mantendo boas características físico-químicas. O trabalho foi realizado com 8 grupos: Cimento experimental com 0,5% de óleo de butiá (B0.5), com 1% (B1), com 2% (B2), com 0,5% de óleo de copaíba (C0.5), 1% (C1) e com 2% (C2), material experimental sem óleos (ME) e a marca comercial RealSeal®(SybronEndo, Orange, CA, USA) (RS), sendo dividido em dois estudos: (1) caracterização do cimento resinoso e incrementação dos óleos de butiá e copaíba com avaliação da sua capacidade antibacteriana e a sua citotoxicidade, segundo os testes de contato direto modificado (TCD) após 1h, 12h e 24h e de viabilidade celular respectivamente; (2) avaliação do seu desempenho físico-químico através dos testes de grau de conversão, espessura de película (EP), tempo de presa, escoamento, radiopacidade todos de acordo com a ISO 6876 (2001) e sorção e solubilidade em água de acordo com a ISO 4049. Os resultados do TCD-1, ME, C0,5, C2 e RS foram estatisticamente semelhantes; para os outros grupos houve uma maior proliferação de bactérias. Em TCD-12, C2 apresentou maior efeito antibacteriano do que os outros grupos experimentais testados. Em TCD-24 todos os grupos experimentais foram equivalentes, apresentando menor atividade antibacteriana. Para citotoxicidade, ME e C2 mostraram viabilidade celular semelhante ao grupo controle ($p = 0,003$). Para os grupos que contém óleo de copaíba, ME, C1 e C2 foram semelhantes o grupo não tratado, e o RS mostrou menor viabilidade celular ($p = 0,003$). Para os testes físico-químicos, os resultados apresentados foram: para o GC, houve uma maior conversão de monômeros em polímeros para os grupos experimentais contendo óleos naturais quando comparados a RS. Para EP, os grupos que contém óleo de Butiá apresentaram valor estatisticamente semelhante entre si ($p = 0,303$). Os grupos que contém óleo de copaíba mostraram que C2 apresentou maior valor que ME e RS ($p = 0,001$). Quanto ao tempo de presa todos os grupos foram estatisticamente semelhantes. Para escoamento, os que apresentaram maior resultado foram B1 e C1. B0.5 e todos os grupos copaíba apresentaram a menor sorção de água e ME B0.5 e B1 a menor solubilidade em água. Para radiopacidade, B2 apresentou os menores valores, seguido pelos outros experimentais e RS, que foi o mais radiopaco. O cimento contendo óleo de copaíba, em todas as concentrações, mostrou radiopacidade semelhante e menor que o RS. Conclui-se que todos os grupos com incorporação dos óleos naturais diminuíram o crescimento antibacteriano e apresentaram redução da citotoxicidade quando comparados a RS, garantindo as propriedades físico-químicas necessárias para cimentos obturadores endodônticos. Porém, copaíba apresentou os melhores resultados antibacterianos com o grupo C2 após 1 e 12h, mostrando-se a formulação mais promissora para esta finalidade.

Palavras chave: medicamentos fitoterápicos; cimento resinoso; materiais obturadores do canal radicular; propriedades físico-químicas; óleos essenciais; *Butia capitata*; *Copaifera sp.*; agentes antibacterianos; *Enterococcus faecalis*; citotoxicidade.

Abstract

REIZNAUTT, Cristiane. **Antibacterial activity and physical chemical properties of a new resin root canal sealer containing natural oils from Butia or Copaiba.** 2017. 56p. Dissertation (Master degree in Dentistry). Graduate Program in Dentistry. Federal University of Pelotas, Pelotas, 2017.

The aim of this study was the development of a dual resin-containing endodontic cement containing phytotherapics that presents antibacterial and non-cytotoxic effect, maintaining good physico-chemical characteristics. The research was carried out with 8 groups: Experimental sealer with 0.5% butia oil (B0.5), with 1% (B1), with 2% (B2), with 0.5% copaiba oil (C0.5), 1% (C1) e com 2% (C2), experimental sealer without oils (ME) and the trademark RealSeal™ (SybronEndo, Orange, CA, USA) (RS) and it was divided into two studies: 1) characterization of the resin sealer and increase of the butia and copaiba oils with evaluation of their antibacterial capacity and their cytotoxicity according to the modified direct contact (TCD) tests after 1h, 12h and 24h and cell viability respectively; (2) evaluation of its physicochemical performance by tests of conversion degree, film thickness, setting time, flow, radiopacity all according to ISO 6876 (2001) and water sorption and solubility in accordance with ISO 4049. The results of TCD-1, ME, C0.5, C2 and RS were statistically similar; The other groups had a higher proliferation of bacteria. In TCD-12, C2 presented higher antibacterial effect than the other experimental groups tested. In TCD-24 all experimental groups were equivalent, presenting lower antibacterial activity. For cytotoxicity, ME and C2 showed statistically similar cell viability to the control group ($p = 0.003$). For groups containing copaiba oil, ME, C1 and C2 were similar to the untreated group, and RS showed lower cell viability ($p = 0.003$). For the physico-chemical tests, the results presented were: for GC, there was a greater conversion of monomers into polymers for the experimental groups containing natural oils when compared to RS. For EP, the groups containing butia oil had a statistically similar value ($p = 0.303$). The groups containing copaiba oil showed that C2 presented higher value than ME and RS ($p = 0.001$). Regarding setting time, all groups were statistically similars. For flow, the ones with the highest result were B1 and C1. B0.5 and all copaiba groups presented the lowest sorption of water and ME B0.5 and B1 had the lowest solubility in water. For radiopacity, B2 presented the lowest values, followed by the other experimental ones and RS, which was the most radiopaque. Sealer containing copaiba oil at all concentrations showed similar and lower radiopacity than RS. It was concluded that all the groups incorporating the natural oils decreased the antibacterial growth and presented reduction of the cytotoxicity when compared to RS, guaranteeing the physico-chemical properties necessary for root canal sealer. However, copaiba presented the best antibacterial results with the C2 group after 1 and 12h, showing the most promising formulation for this purpose.

Keywords: Phytotherapics drougs; resin sealer; root canal filling materials; physicochemical properties; essential oils; *Butia capitata*; *Copaifera sp.*; anti-bacterial agents; *Enterococcus faecalis*; cytotoxicity.

Sumário

1 Introdução

O tratamento endodôntico tem por objetivo final restabelecer a integridade dos tecidos perirradiculares e preservar o elemento dental. Após a descontaminação e limpeza do sistema de canais infectados através do preparo químico-mecânico, é necessário promover adequado vedamento do espaço a fim de evitar a recontaminação (HAMMAD et al., 2008). Portanto, uma obturação satisfatória é determinante para o bom prognóstico da terapia endodôntica (KUMAR et al., 2011; MISHRA et al., 2017). O material obturador endodôntico deve seguir exigências de apresentar princípio biocompatível, insolubilidade, ter estabilidade dimensional, garantir vedação apical, impermeabilidade à umidade, radiopacidade, facilidade no seu manuseio e ser atóxico. (MOZAYENI et al., 2012)

A principal razão para o insucesso endodôntico é a persistência de microrganismos**,** *Enterococcus faecalis* é uma das bactérias mais prevalentes em casos de infecção persistente em dentes tratados endodonticamente (MURAD 2014; POGGIO 2017, HASHEMINIA 2017), e são bactérias diferentes das encontradas em polpa de dentes não tratados (POGGIO 2017). Levando em conta a complexidade anatômica dos canais radiculares, a incapacidade de uma desinfecção total dos condutos e túbulos dentinários e a resistência dos microrganismos (WANG 2017), os materiais utilizados durante o tratamento endodôntico necessitam ter capacidade antibacteriana para auxiliar na redução destes microrganismos durante todas as etapas do tratamento endodôntico, desde o preparo químico-mecânico (PQM), medicação intracanal (MIC) nas intersessões clínicas se existentes, e também, deve estar presente nos cimentos endodônticos utilizados na obturação dos canais radiculares, tendo em vista que após o PQM e MIC o canal não esta estéril. (ØRSTAVIK 2014). Os cimentos endodônticos com características antibacterianas auxiliam a diminuir ou evitar a proliferação bacteriana e também na reparação tecidual apical e periapical (POGGIO 2017; WANG 2017).

O objetivo final do tratamento endodôntico é uma boa obturação do canal radicular para evitar a reinfecção (WANG 2017). Diante disso, outra característica que vem sendo estudada é o tipo de obturação, pois a guta-percha não se liga as paredes dentinárias e mesmo com a presença do cimento pode resultar na ausência de total selamento radicular (MISHRA et al., 2017). Por isso, vem sendo incrementado o uso de cimentos endodônticos resinosos que utilizam monômeros surfactantes, os quais possuem regiões de caráter hidrófobo e outras de caráter hidrófilo, proporcionando assim, íntimo contato com a dentina úmida e garantindo uma maior adesão entre dentina e cimento (ZANCHI, 2010). Visando essas características, há disponível no mercado de produtos odontológicos vários seladores e obturadores de canais à base de resina. Dentre eles, podem ser citados: à base de resina epóxi (AH Plus®, Dentsply, Alemanha); à base de metacrilatos (RealSeal®, SybronEndo, Orange, CA, USA; EndoREZ®, Ultradent, EUA). Destaca-se que dentre os cimentos resinosos, um dos que apresentam polimerização dual é o RealSeal®.

Com a utilização destes materiais e a adesão nos canais radiculares o termo monobloco tornou-se familiar (BELLI et al., 2011; KIM et al., 2010). Isso significaria que o cimento formaria uma suposta massa sólida isenta de espaços vazios, que consiste em diferentes materiais e interfaces obturadoras, com as vantagens pretendidas de melhorar simultaneamente a vedação e a resistência à fratura dos dentes obturados (TAY e PASHLEY, 2007), embora tenham descrito boas características destes materiais na literatura, cumprir a meta ideal de um monobloco no espaço do canal radicular com estes materiais ainda é considerado como um grande desafio (KIM et al., 2010)

Embora haja diferentes agentes endodônticos com características antimicrobianas disponíveis comercialmente, há a preocupação em relação à biocompatibilidade destas substâncias e os seus efeitos tóxicos, alérgicos e mutagênicos (MOZAYENI et al., 2012; ØRSTAVIK 2014). Mesmo que os cimentos endodônticos tenham como função serem utilizados dentro do canal radicular, sempre há alguma extrução do material para o periápice, o que pode ter efeitos biológicos negativos (ARUN 2017). A cicatrização periapical depende, assim, em parte do material utilizado (DIOMEDI 2014). Quando as características do material são um potencial irritante aos tecidos causa uma inflamação periapical somada a já existente patológica, por casa de sua citotoxicidade, o que pode inibir ou atrasar o processo de cicatrização periapical e a vedação apical. (SILVA 2014). Existe uma busca incansável por um cimento endodôntico que não seja tóxico ou que seja menos tóxico. Desta forma, o auxílio de fitoquímicos naturais isolados de plantas, agindo como agentes antibacterianos no cimento são considerados como potenciais alternativos para biocompatibilidade (PALOMBO, 2011).

Os óleos essenciais derivados de plantas aromáticas e medicinais são potencialmente úteis como agentes antimicrobianos e a sua utilização como medicamentos tem sido amplamente reconhecida (BURT, 2004; HOLLEY e PATEL, 2005) por terem eficiência, baixa toxicidade, biocompatibilidade e baixo custo. (TOBOUTI 2017). Dentre estes fitoterápicos, destacaremos os óleos essenciais de "Butiá" e de Copaíba. A árvore *Butia capitata* pertence à família Arecaceae, e produz um fruto bastante consumido no Brasil, o "Butiá" (MAGALHÃES et al., 2008). O óleo essencial desse fruto chama atenção por apresentar um potencial agente antimicrobiano contra bactérias orais (SUN et al., 1988; PERALTA et al., 2013), devido aos ácidos graxos de cadeias longas ou médias presentes em sua composição (FARIA et al., 2008). Além disso, outra vantagem do uso dessa semente do "butiá" como matéria-prima para obtenção de óleos essenciais com características antimicrobianas seria que ela é uma fonte renovável e um material de baixo custo (SZENTMIHALYI et al., 2002). Estudos em adesivos já realizados pelo grupo, demonstram que o butiá apresenta um efeito antimicrobiano eficaz contra bactérias (*Streptococcus mutans*) e lactobacilos.(PERALTA et al., 2013)

A árvore de espécie do gênero *Copaifera* (óleo-resina de "copaíba"), pertence a Família Leguminosae, subfamília Caesalpinioideae (TOBOUTI 2017). O extrato natural obtido do tronco desta árvore além de propriedades antimicrobianas, apresenta ainda característica anti-inflamatória, cicatrizante e analgésica (GARRIDO 2010; TOBOUTI 2017). Dentre suas aplicações farmacológicas na odontologia, pode-se destacar: o seu uso como substituto do eugenol na mistura com o óxido de zinco, produzindo menor irritação (RIBEIRO, 1989); composição de substância obturadora provisória em conjunto com o hidróxido de cálcio (RIBEIRO, 1989); auxiliar no controle da doença periodontal (SOUZA et al., 2011); e na placa dental com ação frente ao *S. mutans* (VALDEVITE, 2007; PIERI et al., 2010; SIMÕES, 2004), também, foi testado em um cimento endodôntico experimental que não era resinoso (GARRIDO 2010).

Ainda não existe um material que preencha todos os requisitos para ser um cimento endodôntico ideal, como: fluir ao longo de toda a superfície da parede do canal, preencher todos os espaços e as lacunas entre o material de obturação e da dentina, aderir tanto dentina quanto na guta-percha (WU et al., 2000), ter efeito antimicrobiano e ser biocompatível (ØRSTAVIK 2014). Por isso, investigar novos cimentos endodônticos continua sendo bastante relevante. Neste contexto, seria promissor o desenvolvimento de um cimento endodôntico fotopolimerizável com óleos naturais, tendo assim, as características do cimento endodônticos resinosos somados as vantagens dos extratos naturais, podendo melhorar a atividade antimicrobiana sem comprometer a sua biocompatibilidade nem suas características físico-químicas. Como não há estudos científicos avaliando a incorporação de extratos de Butiá ou Copaíba na formulação de cimentos endodônticos resinosos, o objetivo deste estudo foi investigar os efeitos da incorporação desses óleos em cimentos experimentais duais, avaliando suas características físico-químicas e biológicas, bem comocomparando o seu desempenho pré-clínico com a referência comercial RealSeal® (SybronEndo, Orange, CA, EUA), cimento resinoso também de polimerização dual.

2 Capítulo 1

Antibacterial potential of new root canal resin sealers containing *Butia capitata* or *Copaifera sp*

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Keywords: essential oils; resin sealer; contact direct test; anti-bacterial agents; *Enterococcus faecalis*; cytotoxicity

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Abstract

Aim: Evaluate the incorporation of vegetable oils with antibacterial potential in experimental root canal resin sealers and your citotoxity.

Methodology: Experimental samples were divided into eight groups (n=3) with different concentrations: butia (*Butia capitata*) 0.5% (B0.5), 1% (B1) and 2% (B2); and copaiba (Copaifera spp.) 0.5% (C0.5), 1% (C1) and 2% (C2) and a control group with no added oils (EM: Experimental Material), and the RealSeal™ (SybronEndo, Orange, CA, USA), used as a trademark comparison to control. Experimental sealers were submitted to biological tests: modified direct contact test after 1h/DCT-1, 12h/DCT-12 and 24h /DCT-24, and cytotoxicity test. Viability celular was analysed statistically using ANOVA and Tukey’s test for multiple comparisons and to determine viable bacteria counts, ANOVA and the Fisher’s test significance difference (LSD) post hoc test for pair-wise means comparisons.

Results: The incorporation of natural oils presented less cytotoxic, mainly copaíba, than trade market. Besides that, all groups presented antibacterial effect, but the incorporation of Copaiba oil at 2% increased the antibacterial effect after 1 and 12 hours ($p<0.05$).

Conclusion: The novel root canal sealers containing natural oils are a great alternative for root-canal filling materialss showing a promising antibacterial capacity and satisfactory cell viability.

1 Introduction

The ultimate goal of root canal therapy is obturation (Mishra *et al.* 2017), on the endodontic therapy after the decontamination and cleaning of infected root canal system through the chemo-mechanical preparation, it is necessary to promote proper sealing of the endodontic space in order to avoid recontamination (Hammad *et al.* 2008).

This treatment does not guarante complete elimination of biofilm or secondary infection, because the anatomical difficulty of the root canals, the inability of a complete disinfection of the conduits and dentinal tubules and the resistance of the microorganisms (Wang *et al.* 2017). The main reason for endodontic failure is the persistence of microorganisms, *Enterococcus faecalis* is one of the most prevalent bacteria in cases of persistent infection in endodontically treated teeth (Murad et al. 2014 & Poggio *et al.* 2017 & Hasheminia *et al.* 2017). The success rate is influenced by the antimicrobial activity of the materials used as irrigating solution, dressing or filling materials, such on the root canal sealer ensuring a good prognostic for endodontic therapy for to restore the integrity of the periradicular tissues and preserve the dental element. (Zaura *et al.* 2009 & Mishra *et al.* 2017)

New root canal methacrylate resin sealers have been introduced in the dental market with the aim to obtain eficcient obturation, because gutta-percha does not adhere to the dentinal walls even with the presence of the root cement, which may result in the absence of total apical sealing. (Mishra *et al.* 2017). This methacrylate resin-based root canal sealers have hydrophobic and other hydrophilic character regions, thus providing an intimate contact with the wet dentin and ensuring a greater adhesion between dentin and cement. (Zanchi *et al.* 2010). This kind of bondable root canal sealer has been aggressively promoted with the highly desirable property of creating monoblocks within the root canal space. The “monoblock” term refers to the scenario wherein the canal space becomes perfectly filled with a gap-free, solid mass that consists of different materials and interfaces, with the purported advantages of simultaneously improving the seal and fracture resistance of the teeth canals (Belli e Kim 2010). Among these cements we can cite the RealSeal™(SybronEndo, Orange, CA, USA) is a root canal resin sealer wich has dual polymerization.

Additionally, although there are various endodontic sealers with antimicrobial features commercially available, there is a concern about the biocompatibility of these substances and their toxic, allergic and mutagenic effects (Mozayeni *et al.* 2012 & Ørstavik 2014). Even if the endodontic cements are intended to be used inside the root canal, there is always some extrusion of the material to the periapice, which may have negative biological effects (Arun *et al.* 2017). Periapical healing depends, therefore, on part of the material used (Diomede *et al.* 2014). When the characteristics of the material are a potential irritant to the tissues causes a periapical inflammation added to the already existing pathological, per cytotoxicity home, which may inhibit or delay the process of periapical healing and the apical seal. (Silva *et al.* 2014). There is a relentless quest for endodontic cement that is non-toxic or less toxic. Thus, the aid of natural phytochemicals isolated from plants, acting as antibacterial agents in the cement are considered as alternative potentials for biocompatibility (Palombo 2011).

Essential oils derived from aromatic and medicinal plants are potentially useful as antimicrobial agents and their use as medicines has been widely recognized, because it has efficiency, low toxicity, biocompatibility and low cost (Tobouti *et al.* 2017).

Among these phytotherapics, we will highlight the essential oils of butia and copaíba. The essential oil of the fruit from *Butia capitata* tree, attracts attention for demonstrating a potential antimicrobial agent against oral bacteria (Faria *et al.* 2008), due to fatty acids of long or medium chains present in its composition (Peralta *et al.* 2013). Research already done in restorative adhesives, demonstrate that the butia features an antimicrobial effect and is effective against total microorganisms, aciduric bacteria, lactobacilli, and *Streptococcus mutans* (Ørstavik 2014). Another natural extract obtained from the trunk of the tree is

Copaifera (oil-resin "copaiba") Belongs Family Leguminosae, subfamily Caesalpinioideae. (Tobouti *et al.* 2017), in addition to antimicrobial properties, also exhibits anti-inflammatory, healing and analgesic characteristics (Tobouti *et al.* 2017).

Among its pharmacological applications, it is worth mentioning: its use as a substitute for eugenol in the mixture with zinc oxide, producing less irritation (Ribeiro 1989); Composition of provisional obturator substance together with calcium hydroxide (Ribeiro 1989); In the control of periodontal disease (Souza *et al.* 2011) and a gel based on copaíba was registered for this purpose (Simões 2004), was also tested in an experimental endodontic cement (Garrido *et al.* 2010)

An ideal endodontic sealant should be antimicrobial and biocompatible, which is still very difficult to find in the market (Gyawali e Ibrahim 2014). There are no scientific studies evaluating the incorporation of Butia and Copaiba oil into endodontic resin sealers. Thus, the aim of this study is to evaluate the antibacterial effect of the experimental sealers containing Butia or Copaiba oil in its composition without compromisse their biocompatibility.

Materials and methods

Material and Reagents

Pasta base	**Percentual em massa (%)**
Bis-EMA 30	30-60
PEG 400	15-35
Exotano 8	10-30
TEGDMA	5-20
CQ	0,1-5
DHEPT	0,1-5
Sílica ñ-silan.	2-20
YT3	15-50

Pasta catalisadora	**Percentual em massa (%)**
Bis-EMA 30	30-60
Exotano 8	20-40
TEGDMA	5-15
Sulf.	0,01-5
PB	0,1-5
BHT	0,01-2
Silica ñ-silan.	2-20
YT3	15-50

The experimental samples were divided into 7 groups with differente concentrations and 1 commercial group, totaling 8 groups (n=3) with different concentrations: butia (*Butia capitata*) 0.5% (B0.5), 1% (B1) and 2% (B2); and copaiba (*Copaifera spp*.) 0.5% (C0.5), 1% (C1) and 2% (C2) and a control group with no added oils (EM), and the RealSeal™(SybronEndo, Orange, CA, USA). Photoactivation procedures were carried out using a light-emitting diode unit (Radii™; SDI, Bayswater, Victoria, Australia) with 800-mW/cm^2 irradiance.

Analysis of the oil by gas chromatography

Copaiba oil: The chromatography analysis was done previously. (Lima *et al.* 2011)
Butia oil: The chromatography analysis was done previously. (Peralta *et al.* 2013)

Modified Direct Contact Test

Enterococcus faecalis (ATCC 4083), was used as the tested microorganism. It was cultured overnight at 37ºC in tryptic soy agar (TSA) plates in an aerobic atmosphere. *E. faecalis* was inoculated in tryptic soy broth (TSB) and the bacterial turbidity was adjusted to an optical density (OD) of 0.5 at 600nm.

Cylindrical specimens of sealer with 6mm in diameter and 1mm thickness (n=3), were curing for 20s with a LED unit (Radii™; SDI, Bayswater, Victoria, Australia). The specimens were placed into the wells of a 96-wells plate. Subsequently, 20µL of bacterial suspension was placed onto the surface of the materials tested. Strain suspensions (20µL) placed in uncoated wells served as non-exposed (positive) controls. Materials incubated in medium without bacteria served as negative controls. All samples were incubated aerobically for 1, 12 and 24h at 37°C in >95% humidity; then 180mL of TSB was added to each of the wells and gently mixed with a pipette for 1 min and with a shaker for 5min. Serial dilutions were prepared in TSB and plated onto TSA and incubated in aerobic environment for 24h at 37°C. The CFU were counted and CFU/mL was calculated (Gyawali e Ibrahim 2014). Experiments were performed in triplicate.

Cytotoxicity Assay

The cytotoxicity test was performed according to ISO 10993-5 (2009). Mouse fibroblasts L929 (20×103/well) were maintained in DMEM medium (Lonza, Basileia, Switzerland) in 96 well plates for 24h. The samples (n = 3) were made in the same form with the previous test. These samples were placed in 24 well plates with 1ml of DMEM at 37°C, pH 7.2 to obtain the eluates and a group without contact with the eluate (untreated cells) being the control group. After 24h, 200uL of eluate from each group were transferred to 96 well plates previously prepared and incubated with cells for 24h. MTT kit (Sigma Aldrich™, MO, USA) was used to assess cell metabolic function for mitochondrial dehydrogenase activity and the absorbance at 540nm was measured using a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, CA, USA).

Statistical analyses

The equality of the variances and the normal distribution of the errors were checked for all tested response variables. Those that did not satisfy these conditions were submitted to transformations as an attempt to fulfill parametric assumptions.

The data of biocompatibility were submitted to ANOVA and Tukey test ($p<0.05$). For microbiological assays, the CFU count data were non-normal. Log^{10} transformation of each CFU count was performed to normalize the data before statistical evaluation due to the high range of bacterial numbers. Then, to determine viable bacteria counts, statistical analyses were performed using one-way ANOVA and the Fisher's test significance difference (LSD) post hoc test for pair-wise means comparisons. All statistical testing was performed using Sigma Stat® for Windows Software®, Version 3.5 (Systat Software, Inc., Point Richmond, CA, USA), using a pre-set alpha of 0.05.

Results

Microbiological effect

The antibacterial effects of the endodontic sealers from modified DCT are presented in Tables 1 and 2. Sealers set for 1, 12 and 24hours showed differences in their activity against *E. faecalis.*

RealSeal™ reduced the number of bacteria during the first 60min of contact, and significantly more bacteria were killed after 12 and 24h ($P < 0.5$), don't presented statistical similarity to any of the. B0.5, B1 and B2 killed more bacteria at 1h and after 24h of contact. The results of butia-based experimental sealers set for 24h were similar to those set for 1h (Table 1).

The groups containing copaiba oils showed that after 1h the group C0.5, C2, and RS demonstrated higher antibacterial effect than C1 group, but C0.5 on 1h don't apresented differene statistic with C1. At 12h, RS group showed the highest antibacterial effect followed by C2; and at 24h the RS continued to exhibit the highest antibacterial activity against *E. faecalis*. (Tables 2).

Cytotoxicity assay

The experimental sealer containing Butia oil at a concentration of 2% showed similar cell viability than control group and EM (p=0,003). For the groups containing copaiba oil, the EM, C1 and C2 were similar to the untreated group (control group). Real Seal was more cytotoxic than the experimental groups (21%). Cytotoxicity data are summarized in Figures 1 and 2.

Discussion

Antibacterial activity of sealers might help to eliminate residual microorganisms that have survived the chemomechanical preparation and thereby improve the success rate of endodontic treatment. One of the challenges in endodontic research has been the lack of standardized in vitro and in vivo protocols for the testing of the antimicrobial effect of sealers. (Mishra *et al.* 2017) The DCT is a quantitative and reproducible method that simulates the contact of the test microorganism with endodontic sealers inside the root canal. What makes possible to evaluate the effect of sealers at various stages of the setting reaction on microbial viability (Zangh *et al.* 2009).

Endodontic sealers with chemical antibacterial agents have been studied, however the cytotoxicity of these materials seems increased. Since medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years (Gyawali e Ibrahim 2014), they could be a good alternative. Plant extracts, phytochemical compounds and essential oils were investigated for their ability to treat or prevent adhesion of oral bacteria to various surfaces(Kouidhi *et al.* 2015). A recent study of our group showed that the incorporation of 1% butia oil into a self-etching adhesive system demonstrated antibacterial activity against *Streptococus mutans* (Peralta *et al.* 2013). In this study, the endodontic sealers containing copaiba oil presented the best results, revealing its antibacterial effect. As already seen in previous studies (Faria et al. 2008 & Peralta *et al.* 2013), where copaiba had an antibacterial effect against other bacteria in the dental biofilm.

E. faecalis are Gram-positive bacteria that increases can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen (Rocas *et al.* 2004). *E. faecalis* overcomes the challenges of survival within the root canal system in several ways. It has been shown to exhibit widespread genetic polymorphisms (Sedgley *et al.* 2004). It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which help it bind to dentin (Hubble *et al.* 2003). It is small enough to proficiently invade and live within dentinal tubules (Love 2001). It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available (Figdor e Sundqvist 2003). *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings based on calcium hydroxide for over 10 days. This may explain why the showed low antibacterial effect, because the bactéria is the most virulent, But all still decreased the bacterial effect.

The cytotoxicity of various filling materials may be investigated using cell culture tests (Heitman *et al.* 2008). The result of the present study was positive in favor of the experimental sealers based on natural extracts. Sealers should not be cytotoxic, preventing the repair process at the periapical region (Gandolfi *et al.* 2008). In the case of copaiba, the authors believe that this fact may be related to the oil properties, such as biological compatibility (Garcia *et al.* 2011) and anti-inflamatory effect (Kobayashi *et al*. 2011). In addition, similar results were reported (Almeida *et al.* 2012), which evaluated the cytotoxic and genotoxic effects of copaiba resin oil and its volatile and resinous fractions. Under the experimental conditions, the authors detected no mutagenic or genotoxic effects, confirming the results of this study. Although the results of cytotoxicity experiments using in vitro cell cultures cannot be directly interpreted in terms of in vivo application, the fact that the experimental sealer with copaiba oil do not cause fibroblast cell line death indicates the possible damage to the compound of the cement biocompatibility.

The experimental endodontic sealer seem to be a viable option in cases where treatment requires an antibacterial activity related to biocompatibility with periapical tissues. The incorporation of essencial oils did not compromise the biocompatibility and decreased the bacterial effect. Besides that, the incorporation of Copaiba oil at 2% increased antibacterial effect after 1 and 12h.

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Tables

Table 1: Modified contact direct test, of experimental material with butia oil (CFU/ml)

	EM mean±SD	B0.5 mean±SD	B1 mean±SD	B2 mean±SD	RS mean±SD	C+ mean±SD
1h	A6.4 ± 0.3b	AB6.55 ± 0.4b	B6.45 ± 0.3b	B6.42 ± 0.3b	A5,95 ± 0,5c	A7.6 ± 0.2a
12h	A6.80 ± 0.6b	A7.06 ± 0.7ab	A7.07 ± 0.6ab	A7.2 ± 0.3ab	B4,49 ± 0,4c	A7.59 ± 0.3a
24h	A6.72 ± 0.8b	B6.29 ± 0.2b	B6.24 ± 1.1b	AB6.75 ± 0.5b	C1,07 ± 1,9c	A7.44 ± 0.3a

Capital letters showed statistical differences in the time, and lower letter showed difference statistical between groups (p>0.05)

Table 2: Modified contact direct test, of experimental material with copaiba oil (CFU/ml)

	EM mean±SD	C0.5 mean±SD	C1 mean±SD	C2 mean±SD	RS mean±SD	C+ mean±SD
1h	A6.4 ± 0.3b	A6.3 ± 0.5bc	A6.61 ± 0.4b	B6.01 ± 0.3c	A5,95 ± 0,5c	A7.6 ± 0.2a
12h	A6.80 ± 0.6b	A6.81 ± 0.9b	A6.99 ± 0.5b	AB6.1 ± 0.1c	B4,49 ± 0,4c	A7.59 ± 0.3a
24h	A6.72 ± 0.8b	A6.76 ± 0.5b	A6.8 ± 0.3b	A6.62 ± 0.7b	C1,07 ± 1,9c	A7.44 ± 0.3a

Capital letters showed statistical differences in the time, and lower letter showed difference statistical between groups (p>0.05)

Figure legends

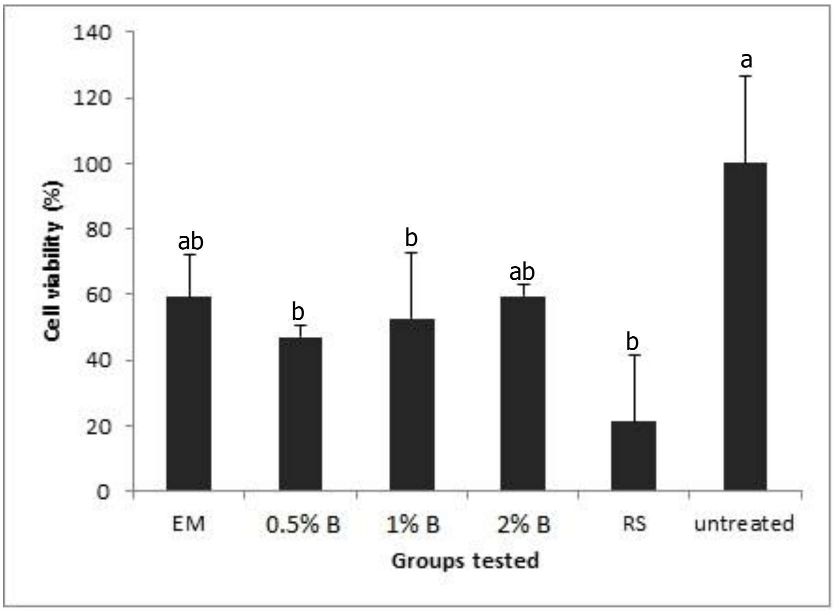

Figure 1. Cell viability (%) after exposure of L929 mouse fibroblast cells to experimental sealers with butia oil (B). Results are expressed as means and standard deviation (SD) according to cell control (untreated group), which it was considered 100%. Different letters indicate statistically significant differences (p <0.05).

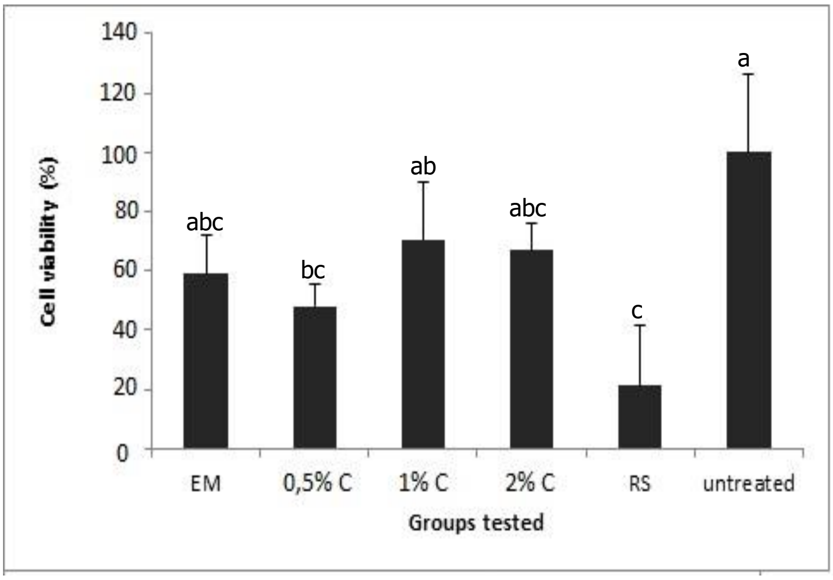

Figure 2. Cell viability (%) after exposure of L929 mouse fibroblast cells to experimental sealer with copaiba oil (C). Results are expressed as means and standard deviation (SD) according to cell control (untreated group), which it was considered 100%. Different letters indicate statistically significant differences (p <0.05).

3 Capítulo 2

Physicochemical properties of the new root canal resin sealers containing promising vegetable oils

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Keywords: essential oils; resin sealer; physicochemical properties; root canal filling materials; phytotherapic drougs

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Abstract

Aim: To evaluate the physicochemical properties in experimental endodontic resin sealers containg butia or copaíba oil.

Methodoloy: Experimental samples were assigned into eight groups with different concentrations: butia (Butia capitata) 0.5% (B0.5), 1% (B1) and 2% (B2); and copaiba (Copaifera spp.) 0.5% (C0.5), 1% (C1) and 2% (C2) and a control group with no added oils (EM), and the RealSeal™ (SybronEndo, Orange, CA, USA), used as a trademark comparison to control. Degree of conversion, film thickness, setting time, flow, water sorption and solubility, and radiopacity of experimental sealers were assessed. Data were submitted to statistical analyses using ANOVA and Tukey's test.

Results: Degree of conversion and film thickness had higher values in the experimental groups containing natural oils. All groups were statistically similar on the setting time. For flow, the ones with the highest result were B1 and C1. B0.5 and all copaiba groups had the lowest water sorption and ME B0.5 and B1 had the lowest solubility in water.

The incorporation of Butia or Copaiba oil did not modify the physicochemical properties of the experimental filling materials ($p<0.05$).

Conclusion: The novel endodontic sealers containing natural oils are a great alternative for endodontics, due to their good physico-mechanical characteristics when compared to a commercially available material (RealSeal™).

Introduction

There are several endodontic cements on the market, they are classified on five groups according your chemical compositions: zinc-oxide–eugenol sealers, sealers containing calcium hydroxide, resin-based sealers, glass–ionomer-based sealers and silicone-based sealers. (Garrido *et al.* 2010)

The resin sealers emerged for improve the role for guta-percha, because this not completely fill the root canal. (Mishra *et al.* 2017). Among , they can be mentioned: based on epoxy resin (AH Plus™, Dentsply, Germany); Based on methacrylates (RealSeal™, SybronEndo, Orange, CA, USA and EndoREZ™, Ultradent, USA). That among the resin cements, what has dual polymerization is Real Seal™. This methacrylate resin root canal sealers have hydrophobic and other hydrophilic character regions, thus providing an intimate contact with the wet dentin and ensuring a greater adhesion between dentin and cement. (Zanchi 10). This kind of bondable root canal sealer has been aggressively promoted with the highly desirable property of creating supposed monoblock within the root canal space. The "monoblock" term refers to the scenario wherein the canal space becomes perfectly filled with a gap-free, solid mass that consists of different materials and interfaces, with the purported advantages of simultaneously improving the seal and fracture resistance of the teeth canals (Belli 2010). Among these cements we can cite the Real Seal™ (SybronEndo, Orange, CA, USA) is a root canal resin sealer wich has dual polymerization.

Products in the area of phytotherapy have been studied for dental applications. The tree *Butia capitata* belongs to the family Arecaceae, and produces a very consumed fruit in Brazil, the "butia" (Magalhães *et al.* 2008) it attracts attention for demonstrating a potential antimicrobial agent against oral bacteria (Faria *et al.* 2008), due to fatty acids of long or medium chains present in its composition (Peralta *et al.* 2013). Research already done in restorative adhesives, demonstrate that the butia features an antimicrobial effect and is effective against total microorganisms, aciduric bacteria, lactobacilli, and *Streptococcus mutans* (Peralta *et al.* 2013). Another natural extract obtained from the trunk of the tree is *Copaifera* (oil-resin "copaiba") Belongs Family Leguminosae, subfamily Caesalpinioideae. (Tobouti *et al.* 2017), in addition to antimicrobial properties, also exhibits anti-inflammatory, healing and analgesic characteristics (Tobouti *et al.* 2017).

Among its pharmacological applications, it is worth mentioning: it use as a substitute for eugenol in the mixture with zinc oxide, producing less irritation (Ribeiro 1989); Composition of provisional obturator substance together with calcium hydroxide (Ribeiro 1989); In the control of periodontal disease (Souza *et al.* 2011), and a gel based on copaíba was registered for this purpose (Simões 2004), was also tested in an experimental endodontic cement (Garrido *et al.* 2010).

The root canal sealer must comply with the requirements of presenting biocompatible principle, insolubility, dimensional stability, ensure apical seal, impermeability to moisture, radiopacity and ease in its handling. (Mozayeni *et al*. 2012)

In order to launch a new product on the market, it must comply with the ANSI/ADA's specification number 57 (1984) evaluated for its physicochemical properties.

The aim of this study was to compare an experimental root canal resin sealer based on Butia or Copaiba oil with commercial resin sealer Real Seal™(SybronEndo, Orange, CA, USA) wich has dual polimerization, second on terms of physicochemical properties required by the ANSI/ADA.

Materials and methods

Material and Reagents

Pasta base	**Percentual em massa (%)**
Bis-EMA 30	30-60
PEG 400	15-35
Exotano 8	10-30
TEGDMA	5-20
CQ	0,1-5
DHEPT	0,1-5
Sílica ñ-silan.	2-20
YT3	15-50

Pasta catalisadora	**Percentual em massa (%)**
Bis-EMA 30	30-60
Exotano 8	20-40
TEGDMA	5-15
Sulf.	0,01-5
PB	0,1-5
BHT	0,01-2
Silica ñ-silan.	2-20
YT3	15-50

The experimental samples were divided into 7 groups with differente concentrations and 1 commercial group, totaling 8 groups (n=3) with different concentrations: butia (*Butia capitata*) 0.5% (B0.5), 1% (B1) and 2% (B2); and copaiba (*Copaifera spp*.) 0.5% (C0.5), 1% (C1) and 2% (C2) and a control group with no added oils (EM), and the RealSeal™(SybronEndo, Orange, CA, USA). Photoactivation procedures were carried out using a light-emitting diode unit (Radii™; SDI, Bayswater, Victoria, Australia) with 800-mW/cm^2 irradiance.

Analysis of the oil by gas chromatography

Copaiba oil: The chromatography analysis was done previously. (Lima *et al.* 2011)

Butia oil: The chromatography analysis was done previously. (Peralta *et al.* 2013)

Degree of conversion by RT-FTIR spectroscopy

The C double bond; length as m-dash C conversion of the experimental material (n = 3) was determined using Fourier transform infrared (FTIR) spectroscopy (Prestige 21 spectrometer Shimadzu Corporation, Kyoto, Japan), equipped with an attenuated total reflectance attachment incorporating a horizontal diamond crystal (PIKE Technologies, Madison, WI, USA). The LED (Radii® Curing Light, SDI, Baywater, Victória, Austrália) curing unit was rigidly held in position, enabling standardization of the distance between the fiber tip and the top of the sample at 2mm. Infrared analysis was performed at a controlled room temperature of 23˚C (±2˚C) and 60% (±5%) relative humidity. Approximately 5mg of each sample was dispensed directly onto the diamond crystal, after which the degree of conversion was evaluated. The spectra of the uncured and cured (after 20s of photo-activation) experimental materials were acquired between 1,690–1,575cm^{-1}, averaging 12 scans at the 4cm^{-1} resolution transmission mode, to provide a single spectrum. Spectra of each unpolymerized material were also captured (i.e., a single scan was also collected immediately prior to light curing). The extent of the unreacted aliphatic carbon double bonds (% C=C) was determined from the ratio of the absorbance intensities of the aliphatic C=C (peak height at 1,637cm^{-1}) to that of an aromatic C=C absorbance of the internal standard component (1,608cm^{-1}), both before and after curing the specimen.

Film thickness

Film thickness was evaluated according to ISO 6876 (2001). Square glass plates (thickness 5mm) stacked in contact was measured with a digital caliper accurate to 1μm (Mitutoyo®, São Paulo, Brasil). A constant volume of the sealers (0.5mL) was placed centrally over a first plate, them another plate was placed on top of that after 180s±10, a constant load of 150N was applied via the top plate for more 7min. The loading system was released and the combined thickness of the two glass plates and the sealer film was measured. Film thickness (μm) was recorded as the difference between the readings (n = 3).

Setting time

For this test, cylindrical metal matrices with internal diameter of 10mm and thickness of 2mm were used, where the specimens were made (n = 3 for each material). The matrices were fixed on a glass plate with the aid of utility wax. Subsequently, the cement was manipulated and inserted into the matrix until it filled. After 120±10s of the start of the mixture, the set was placed on a metal block of dimensions 8x20x10 mm, placed in a sealed plastic container, kept at a constant temperature of 37 ºC. Subsequently, a 100g Gillmore type needle with a 2mm active tip was placed in contact with the horizontal surface of the material. This procedure was repeated at regular time intervals of 60s until no indentations occurred in the material tested. The elapsed time from the end of the cement mixing until the indentations were no longer visible was considered the holding time of the material.

Flow

One volume of 0.05±0.005 ml of the cement was prepared and dispensed onto a glass plate of dimensions 40x40mm of approximately 5 mm thickness with the aid of a 1ml syringe (n = 3 for each group of material). After 180±5s from the start of the manipulation, another glass plate weighing about 20g was carefully positioned on the cement and, on this second plate, a weight of 100g, thus totaling 120±2g. Ten minutes after the start of the mixing the weight was removed. The formed cement disc was measured in its largest and smallest diameter by means of a digital caliper with an accuracy of 0.01mm. For the analysis of the results, the mean of the largest and smallest diameter measurements was used. In order for the cement to conform to the ISO specification, the average diameter of the discs should be equal to or greater than 20mm.

Water Sorption and Solubility

This test was carried out in accordance with the specifications of ISO 4049, specific for resin-based sealing materials. Samples was maded using a 15mm diameter by 1 mm thick die. The material was inserted into the matrix with an overlapping polyester strip and glass plate at both the base and the top (n=5). Then, the photoactivation was performed with 9 incidences of 40s each, in each face of the sample, using light curing device with LED source and intensity ≥1,100mW/cm^2 (Radii-cal®, SDI, Australia). After photoactivation, the specimens was transferred to a desiccator and stored at 37°C for 24h. After this period, the samples was weighed into an analytical balance with an accuracy of 0.00001g (Shimadzu AUW220D®, Shimadzu, Japan). The cycle was repeated until a constant mass m1 is obtained, that is, until the mass loss of each specimen is not greater than 0.1mg in each 24h period. The specimens was immersed in distilled water at 37°C for 7 days on a metal shelf. After 7 days, the specimens was removed and the surface dry with absorbent paper and light air for 10s. Then the m2 was obtained. After this weighing, the specimens was reconditioned to the constant mass in the desiccator, using the already described cycle, until obtaining a constant mass, m3. The percentage of water sorption (Wsp) and solubility (Wsl) was calculated from the difference between the masses, where:

$$\text{Wsp} = \frac{m2 - m3}{m1} \qquad \text{Wsl} = \frac{m1 - m3}{m1}$$

Radiopacity

The radiopacity was evaluated according to ISO 6876 (2001). Silicone molds (5mm internal diameter and 1.0mm high) were used for sample preparation (n=3). Three samples per sealer were produced (Mitutoyo®, São Paulo, Brasil), stored in closed receptacles in an incubator at 37ºC until complete setting. Thereafter, the specimens were placed onto occlusal radiographic film (Insight; Kodak Comp., Rochester, NY, USA) alongside a graduated aluminum stepwedge with a purity greater than 98%, 50x20mm and a thickness varying step-shaped every 0.5mm (up to 9mm). The x-ray exposures were made using a Spectro II x-ray unit (Procion Ion 70x, Ribeirão Preto, SP, Brazil) with a 2.5mm aluminum filter added. The tube voltage was 70 kV and the current 8mA. The exposure time was 0,36s with a constant source-to-film distance of 300mm. The exposed films were processed manually by the time/temperature method.

The radiographs were digitized using a desktop scanner (Expression 636(r); Epson) and then saved in TIFF format. Image J™ (RSB, USA) was used to analyze each image by the intensity histogram of tone scales in the "light channel" to obtain an average value of brightness intensity for each specimen. Contrast and brightness of each image were standardized at 40 and 30, respectively. The radiographic density of the materials was compared with the radiopacity of different thicknesses of the aluminum stepwedge.

Statistical analyses

The equality of the variances and the normal distribution of the errors were checked for all tested response variables. Those that did not satisfy these conditions were submitted to transformations as an attempt to fulfill parametric assumptions.

The data of tests were submitted to ANOVA and Tukey test ($p<0.05$) and Kruskal Wallis and for butia and copaiba's sorption and solubility. All statistical testing was performed using Sigma Stat® for Windows Software®, Version 3.5 (Systat Software, Inc., Point Richmond, CA, USA), using a pre-set alpha of 0.05.

Results

For the degree of conversion are demonstrated in Tables 1 and 2. For both groups containing oils, there was a slight increase in the degree of conversion, since the RS showed the lowest degree of conversion.

The results of the film thickness for the group containing butia oil (Table 1), statistically similar values were detected in all groups ($p=0.303$). For the groups containing copaiba oil (Table 2), than C2 presented higher value than EM and RS ($p=0.001$).

For Setting time the groupf (Table 1 and 2), not presented statistically different for all groups.

The results for flow, the results were not statistically different for all groups (Table 1 and 2).

For sorption with groups butia oil (Table 1) there is a statistically significant difference ($P=0.001$) and the best result it was B0.5, B1 and B2. For sorption with oil copaíba (Table 2), all groups containing butia presented the best results ($P=<0.001$).

For solubility with groups copaíba oil the best result it was EM and groups with copaiba oil (P=0.006) (Table 2) and with groups butia oil (Table 1) the best results it was B0.5, B1 and EM (P=<0.001).

The radiopacity values for each sealer material are displayed in the Tables 1 and 2. For the groups containing butia oil, B2 presented the lowest radiopacity values, followed for the other experimental materials and RS, which was the most radiopaque sealer. Differences were found between all groups ($p<0,05$ in comparison to B2). For the experimental sealers containing Copaiba oil, all the concentrations tested showed similar radiopacity, but the RS presented the highest radiopacity ($p<0,05$) (Table 2).

Discussion

The ideal root canal sealer should be dimensionally stable, and possess good antimicrobial activity and low toxicity toward the surrounding tissue (Ørstavik 2014).

The incorporation of natural oils in the experimental sealer did not significantly influence its physical and chemical properties, can be the small amount of essential oil that has been added.

For the degree of conversion all experimental materials presented values above 20%. This is important because it is known that the material to be polymerized in a longer time. Besides being photopolymerizable it presents chemical activation, which ensures a greater connection C=C. The groups that presented the highest degree of conversion as c2 and all with butia, they also present the least time of chemical prey with the trademark, which may lead to believe that the highest polymerization is actually photopolymerized of the experimental material, so the chemical prey time was higher.

The working time test did not realized because the setting time is long for all materials and they are dual polimerization.

All experimental sealers presented flow than RS, what favors it to flow along the whole channel wall filling in gaps of dentin, wich is a prerequisite for root canal sealers. (Wu *et al*. 2000). the c2 group had viscosity above the allowed but a good antibacterial effect to supply the low viscosity.

The oil of Butia and Copaiba contain long hydrophobic chains and carboxyl hydrophilic groups, they are insoluble on water, may have contributed to results for less solubility (Peralta et al. 2013). But, all material with natural reagentes can degradation of these components over time which can justifies solubility (even if little, but present). For sorption, the materials are of dual polymerization, while the masses are still stabilized there may still be some chemical reaction until the final polymerization, like B0.5 and all groups with copaiba oil.

The RS presented the highest radiopacity value and was slightly superior to 7mm Al, while the radiopacity of experimental materials corresponded to around 3mm Al. According to the ANSI/ADA's Specification Number 57, the radiopacity of a root canal sealer material must be equivalent to at least 3mm Al. An increasing of the radiopacifier could alter the biocompatibility, creating a cytotoxic material as well a decrease of the degree of conversion and depth of cure. For radiopacity away, bovine enamel and dentin were used. Although swine teeth presented always lower values of radiodensity when compared to human tooth, bovine ones can substitute in a more reliable manner human teeth in studies that employ the analysis of radiodensity (Fonseca *et al.* 2004). The material must have high radiopacity so that it can be visualized in radiographic examination, differentiating it from the degree of radiopacity of the dentin that is smaller, in order to be identified.

The experimental sealer based on copaiba or butia oil presented satisfactory results in the physicochemical tests required by the ADA.

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Tables

Table 1. Degree of conversion, film thickness, radiopacity, flow, setting time, whater sorption and whater solubility of experimental endodontic sealer with butia oil (mean ± SD)

	M0	B0.5	B1	B2	RS
Degree conversion (%)	22.67±7ab	52.42±4.9a	49.53±13.3a	35.63±11.7a	5.2±1.1b
Film tickness (mm)	19±6a	23.67±8.1b	23±3.6a	14.33±1.5a	21.67±6.1a
Radiopacity (mm)	3.44±0.1b	3.27±0.1b	3.23±0.1b	2.68±0.1c	7.83±0.2a
Flow	20.09±0.01a	21.3±0.7a	21.5±0.2a	20.09±0.1a	20.9±0.005a
Setting time (h)	30.01±0.01a	36.01±0.01a	36.01±0.01a	36±0.01a	24±0.01a
Water Sorption	16.2±0.4a	-0.2±0.07c	0.07±0.04bc	0.2±0.1abc	12.8±0.1ab
Water Solubility	0.9±0.2c	1.1±0.9c	1.6±0.1c	6.9±1.1b	14.3±0.6a

Different lower letters are significantly different (p<0.05)

Table 2. Degree of conversion, film thickness, radiopacity, flow, setting time, whater sorption and whater solubility of experimental endodontic sealer with copaiba oil (mean ± SD)

	M0	C0.5	C1	C2	RS
Degree conversion (%)	22.67±7b	20.86±7.2b	29.7±7.4b	40.06±7.7a	5.2±1.1c
Film tickness (mm)	19±6a	38±10.2b	37±6b	58±3a	21.67±6.1a
Radiopacity (mm)	3.44±0.1b	2.74±0.6b	3.25±0.7b	3.01±0.7b	7.83±0.2a
Flow	20.09±0.01a	23.7±2.7a	24.3±2.9a	21.5±2.1a	20.9±0.005a
Setting time (h)	30.01±0.01a	36.01±0.01a	36.01±0.01a	36±0.01a	24±0.01a
Water Sorption	16.2±0.4a	-0.4±0.1c	-0.2±0.3c	-0.3±0.1c	12.8±0.1b
Water Solubility	0.9±0.2b	7.1±4ab	6.1±5.5ab	5.7±4.2ab	14.3±0.6a

Different lower letters are significantly different (p<0.05)

4 Conclusão

O objetivo do trabalho de criar um novo cimento endodôntico composto por extratos naturais cumpriu-se com bons resultados. A incrementação de óleos naturais de butia e copaíba diminuíram o crescimento bacteriano frente ao contato direto com *E. faecalis*, apresentaram menor citotoxicidade do que a marca comercial testada como comparativa (RealSeal®(SybronEndo, Orange, CA, USA)) e cumpriram as especificações exigidas pela ANSI/ADA's quanto ao seu papel de cimento endodôntico resinoso.

Diante dos óleos e concentrações testados, o cimento resinosoque mais se destacou foi o cimento experimental acrescido de copaíba 2%, grupo no qual teve a menor redução do crescimento bacteriano. Frente a isso, seria o material de escolha mais promissora para a sequência dos trabalhos.

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Apêndices

Apêndice A – Nota de Dissertação

Nota da Dissertação

Atividade antibacteriana e propriedades físico-químicas de um novo material obturador endodôntico resinoso contendo óleos de Butiá ou Copaíba

Antibacterial activity and physico-chemical properties of a new resin root canal sealer containing natural oils from Butiá or Copaíba

A presente dissertação de mestrado desenvolveu novos cimentos obturadores endodônticos resinosos que contêm, em sua formulação, diferentes concentrações de um óleo natural, óleo de butiá ou copaíba. Foi possível ser comparado com uma marca comercial, Real Seal (SybronEndo, Orange, CA, EUA), devido a ser o único cimento endodôntico que fosse resinoso e também de polimerização dual, inclusive, esta referência comercial não é vendida no Brasil, estimulando o desenvolvimento de um produto deste tipo de origem nacional.. Desta forma foi possível avaliar o comportamento do novo material desenvolvido com um material semelhante já a venda no mercado, e ainda comparar com o material desenvolvido sem a presença dos óleos vegetais. Já existem diversos cimentos endodônticos no mercado, mas sempre há preocupação de seus efeitos alérgicos, tóxicos e mutagênicos. Frente a isso a necessidade de criar um cimento que tivesse bom efeito antibacteriano, comportando-se biocompatível e apresentando as porpriedades físico-químicas necessárias para um material endodôntico resinoso. Há poucos estudos na área endodôntico com produtos naturais, então a curiosidade da boa aplicação destes fitoterápicos para esta aplicação. Através dos estudos realizados e bons resultados, onde todos os grupos do cimento experimental apresentaram efeito antibacteriano, baixa citotoxicidade e bons resultados físico-mecânicos frente aos testes realizados, o presente trabalho serve como primeira etapa do desenvolvimento deste material, o qual ainda pode passar por mais testes laboratoriais seguidos de estudos em modelo animal e avaliações clínicas.

Campo da pesquisa: Materiais Odontológicos

Candidata: Cristiane Reiznautt. Cirurgiã-dentista pela Universidade Federal de Pelotas (2015)

Data da defesa e horário: 25/08/2017 às 9:00h.

Local: Auditório do Programa de Pós-graduação em Odontologia da Universidade Federal de Pelotas. 5º andar da Faculdade de Odontologia de Pelotas. Rua Gonçalves Chaves, 457.

Membros da banca: Prof. Dra. Nadia , Prof. Dra. Hellen Lacerda e Prof. Dra. Adriana Silva (Suplente)

Orientador: Prof. Dr. Rafael Guerra Lund

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Apêndice B – Súmula do currículo do candidato

Súmula do currículo

Cristiane Marcant Reiznautt nasceu em 13 de janeiro de 1992, em Pelotas, Rio Grande do Sul. Completou o ensino fundamental em escola privada na mesma cidade (Escola São Francisco de Assis) e médio também em Escola privada na mesma cidade (Colégio São José). No ano de 2010 ingressou na Faculdade de Odontologia da Universidade Federal de Pelotas (UFPel), tendo sido graduada cirugiã-dentista em 2015. No segundo semestre do mesmo ano ingressou no Mestrado do Programa de Pós-graduação em Odontologia da Universidade Federal de Pelotas (UFPel), área de concentração Dentística, sob orientação do Prof. Dr. Rafael Guerra Lund. Durante o período de graduação atuou como monitora do laboratório UPC III, e trabalhou como Iniciação Científica nos laboratórios CDC- Bio e de Microbiologia sob orientação do professor mencionado acima e co-orientação da doutoranda Sonia Peralta. Durante o período de mestrado seguiu sua linha de pesquisa (Materiais nanoestruturados, desenvolvimento, controle e caracterização de biomateriais), desenvolvendo seu trabalho nos mesmos laboratórios.