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Citotoxicidade e capacidade de limpeza de soluções com potencial para uso em endodontia

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Citotoxicidade e capacidade de limpeza de soluções com potencial para uso em endodontia

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Orientadora: Profa. Dra. Gisele Faria

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Citotoxicidade e capacidade de limpeza de soluções com potencial para uso em endodontia

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Dedico este trabalho:

A Deus, o sendeiro da bondade e justiça, e da mais sublime sabedoria. Na sua eterna misericórdia, sei que não existem impossíveis.

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“Considero feliz aquele que quando se fala de êxito busca a resposta em seu trabalho”

Ralph Waldo Emerson

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RESUMO

O hipoclorito de cálcio [$Ca(OCl)_2$] e o cloridrato de octenidina (OCT) têm sido estudados como potenciais soluções irrigadoras endodônticas alternativas ao hipoclorito de sódio (NaOCl) ou à clorexidina (CHX). Os objetivos deste estudo foram avaliar a citotoxicidade do $Ca(OCl)_2$ e do OCT (Publicação 1), e o efeito do OCT na limpeza do canal radicular (Publicação 2). Para a avaliação da citotoxicidade, foram utilizadas células do ligamento periodontal humano (hPDL) e fibroblastos da linhagem L929. As células foram expostas a diferentes diluições das soluções de $Ca(OCl)_2$ a 2,5 e 5%, OCT a 0,1%, NaOCl a 2,5% e CHX a 2%. A viabilidade celular foi avaliada por meio dos ensaios do metil-tiazol-tetrazólio (MTT) e do vermelho neutro (NR), e a migração celular pelo teste de cicatrização. A análise estatística foi efetuada empregando ANOVA de dois fatores e teste de Bonferroni (α=0,05). Os ensaios MTT e NR mostraram, nas células do hPDL, que o OCT a 0,1% foi menos citotóxico ($P<0,05$), seguido da CHX a 2% e do $Ca(OCl)_2$ a 2,5% ($P<0,05$). Não houve diferença significativa entre NaOCl a 2,5% e $Ca(OCl)_2$ a 5% ($P>0,05$), sendo que estas soluções apresentaram maior citotoxicidade que as demais. Em L929, o resultado foi similar, exceto que não houve diferença significativa entre CHX a 2% e $Ca(OCl)_2$ a 2,5% ($P>0,05$). No teste de cicatrização, tanto nas células do hPDL quanto L929, a migração celular do OCT a 0,1%, CHX a 2%, $Ca(OCl)_2$ a 2,5% foi significativamente maior do que o $Ca(OCl)_2$ a 5% e NaOCl a 2,5% às 24 horas ($P<0,05$). Para a avaliação da capacidade de limpeza do OCT, foram utilizados 50 dentes unirradiculados humanos extraídos que foram distribuídos aleatoriamente em 5 grupos (n=10) de acordo com as soluções irrigadoras utilizadas no preparo biomecânico do canal radicular: G1, OCT a 0,1%; G2, CHX a 2%; G3, NaOCl a 2,5%; G4, OCT + ácido etilenodiaminotetracético (EDTA) a 17% e G5, NaOCl a 2,5% + EDTA a 17%. A *smear layer* foi avaliada em microscópio eletrônico de varredura utilizando sistema de escores. Os resultados foram analisados pelos testes de Kruskal-Wallis e Dunn (α = 0,05). Não houve diferença significativa entre os grupos OCT, CHX e NaOCl ($P>0,05$), e esses grupos apresentaram maiores valores de *smear layer* que os grupos NaOCl + EDTA e OCT + EDTA ($P<0,05$). Não houve diferença significativa entre os grupos NaOCl + EDTA e OCT + EDTA ($P>0,05$). Pode-se concluir que, nas linhagens celulares estudadas, o OCT a 0,1% foi a solução menos citotóxica, e que o $Ca(OCl)_2$ a 2,5% e a 5% apresentaram citotoxicidade menor ou similar ao NaOCl a 2,5%, respectivamente. O OCT, utilizado como solução irrigadora única, não apresentou capacidade de limpeza e deveria ser usado em associação com EDTA como irrigante final para se obter remoção da *smear layer*.

Palavras-chave: Endodontia. Microscopia eletrônica de varredura. Teste de materiais. Tratamento do canal radicular.

Coaguila-Llerena EH. Cytotoxicity and cleaning capacity of solutions with potential to be used in endodontics [Dissertação]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

ABSTRACT

Calcium hypochlorite [$Ca(OCl)_2$] and octenidine hydrochloride (OCT) have been studied as alternative irrigating solutions to sodium hypochlorite (NaOCl) or chlorhexidine (CHX). The objectives of this study were to evaluate the cytotoxicity of $Ca(OCl)_2$ and OCT (Publication 1), and the effect of OCT on root canal cleaning (Publication 2). For cytotoxicity evaluation, human periodontal ligament (hPDL) cells and permanent cell line of mouse fibroblasts L929 were used. The cells were exposed to different doses of different solutions: 2.5% and 5% $Ca(OCl)_2$, 0.1% OCT, 2.5% NaOCl and 2% CHX for 10 minutes. Cell viability was assessed by methyl-thiazol-tetrazolium (MTT) and neutral red (NR) assays, and cell migration was determined by the scratch assay. Statistical analysis was performed by two-way ANOVA and Bonferroni tests ($\alpha=0.05$). The MTT and NR assays revealed that 0.1% OCT was less cytotoxic in hPDL cells ($P<0.05$), followed by 2% CHX and 2.5% $Ca(OCl)_2$ ($P<0.05$). There was no significant difference between 2.5% NaOCl and 5% $Ca(OCl)_2$ ($P>0.05$), but these solutions showed greater cytotoxicity than the others. The result was the same for L929 cells, except that there was no significant difference between 2% CHX and 2.5% $Ca(OCl)_2$ ($P>0.05$). Scratch assay in L929 and hPDL cells showed that cell migration of 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$ groups was higher than 5% $Ca(OCl)_2$ and 2.5% NaOCl groups at 24 hours ($P<0.05$). For evaluation of OCT cleaning capacity, fifty human unirradicular extracted teeth were randomly distributed in 5 groups (n=10) according to irrigant solutions which were used during root canal preparation: G1, 0.1% OCT; G2, 2% chlorhexidine (CHX); G3, 2.5% sodium hypochlorite (NaOCl); G4, OCT + 17% ethylenediaminetetraacetic acid (EDTA) and G5, 2.5% NaOCl + 17%. The smear layer was evaluated using a score system and the data were analyzed by Kruskal-Wallis and Dunn ($\alpha=0.05$). In all root canal thirds there was no significant difference between OCT, CHX and NaOCl groups ($P>0.05$), and these groups showed higher smear layer values than NaOCl + EDTA and OCT + EDTA groups ($P<0.05$). There was no significant difference between NaOCl + EDTA and OCT + EDTA groups ($P>0.05$). In conclusion, in tested cell lines, 0.1% OCT had lower cytotoxicity than CHX, $Ca(OCl)_2$ and NaOCl. $Ca(OCl)_2$ at concentrations of 2.5% and 5% showed lower or similar cytotoxicity to 2.5% NaOCl, respectively. The OCT used as a single root canal irrigant presented poor cleaning capacity and could be used in association with a final irrigation with EDTA to obtain smear layer removal.

Keywords: Endodontics. Scanning electron microscopy. Materials testing. Root canal therapy.

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1 INTRODUÇÃO

O principal objetivo do tratamento endodôntico de dentes com necrose pulpar é a redução da infecção microbiana do sistema de canais radiculares[1,2], sendo que a solução irrigadora empregada no preparo biomecânico tem um papel importante para atingir este objetivo[3].

O hipoclorito de sódio (NaOCl) é a solução irrigadora mais comumente utilizada devido a sua atividade antimicrobiana e capacidade de dissolução tecidual[2,4,5], sendo considerado “padrão ouro”[2,6]. No entanto, o NaOCl altera negativamente as propriedades mecânicas da dentina, tais como microdureza, módulo de elasticidade, resistência à flexão e à fadiga[7], e pode reduzir a força de união dos cimentos endodônticos[8] e de alguns materiais adesivos à dentina[7,9]. Além disso, o NaOCl não propicia adequada remoção da *smear layer* da superfície dentinária[2], e pode interagir com outros irrigantes endodônticos, ocasionando efeitos deletérios à dentina radicular[10,11].

Em relação à toxicidade, o NaOCl em concentrações elevadas é altamente irritante quando em contato com tecidos periapicais[12,13].

Outra consideração é o seu potencial efeito prejudicial sobre o processo de regeneração pulpar, do qual participam as células tronco da papila apical – SCAPs[14]. Quando utilizado a 0,5%, 1,5% e 3%, o NaOCl reduz aproximadamente 37% a sobrevivência das SCAPs, e a 6% tem um efeito negativo pronunciado na sobrevida e na diferenciação das SCAPs[15]. Ele também reduz a liberação do fator de crescimento transformador beta 1 (TGF-ß1) da dentina[16], que por sua vez age como um quimiotático para células progenitoras[17] e induz a diferenciação celular e a síntese da matriz[18,19].

A CHX é amplamente utilizada na Odontologia devido a sua eficácia sobre micro-organismos gram-positivos, gram-negativos, aeróbios, anaeróbios facultativos, leveduras e vírus. Outra favorável e importante característica da CHX é sua substantividade, que permite efeito residual prolongado e não promove resistência microbiana[20]. Por outro lado, a CHX não dissolve tecidos orgânicos[21], não remove a *smear layer*[22], e quando empregada como solução irrigadora endodôntica, ela interage com o NaOCl residual no canal radicular, resultando na formação de um precipitado que pode interferir na capacidade de selamento do cimento endodôntico e induzir mudança de cor do dente[2,23-25]. Outro efeito adverso que a CHX pode apresentar é toxicidade, o que poderia prejudicar o reparo dos tecidos periapicais[26,27]. Quando injetada no espaço subplantar da pata de camundongos a CHX, nas concentrações de 0,5 e de 1%, induz efeitos tóxicos severos como necrose da epiderme, derme e tecido subcutâneo em associação com uma resposta inflamatória reacional. Além disso, em cultura de fibroblastos, a CHX induz apoptose em concentrações mais baixas e necrose em concentrações mais elevadas e induz ao estresse celular[26,28].

A procura por soluções irrigadoras alternativas ao NaOCl e à CHX é focada em substâncias que possuem efeito antimicrobiano, de dissolução tecidual, além de não ocasionar danos à estrutura dentária e ser biocompatível.

O Octenisept® (Schülke & Mayr, Nordersdedt, Germany) é um agente antimicrobiano utilizado para antissepsia de queimaduras, de feridas cirúrgicas, da pele e de mucosas[29,30] e atualmente representa uma alternativa à CHX, idopovidona (PVPI) e triclosan[31]. O Octenisept® é composto por cloridrato de octenidina (OCT) e fenoxietanol, um derivado etanol, que serve como conservante[29] e sinergicamente melhora a atividade antimicrobiana do OCT[32].

A molécula do OCT [N, N '- (1,10-decanodiol 1 [4H] -piridinil-4-ilideno) bis (1 octanamine) dicloridrato] pertence as bipiridinas e carrega dois centros catiônicos ativos por molécula. O OCT apresenta amplo espectro de ação frente a bactérias gram-positivas, gram-negativas, fungos e vírus[29,33], sendo o seu espectro de ação comparável ao da CHX[34]. A atividade bactericida / fungicida do OCT se dá pela sua interferência na parede e membrana celular dos micro-organismos[29,35]. O OCT não é absorvido pela pele, não apresenta efeito sistêmico após sua aplicação na mucosa bucal[36] e não há registros na literatura de efeitos carcinogênicos ou mutagênicos[29].

Tem sido mostrado na literatura que o OCT mantém a sua eficácia antimicrobiana frente a *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecium* e *Candida albicans* na presença de material orgânico[37]. Esta é uma propriedade importante, uma vez que material orgânico presente no sistema de canais radiculares pode diminuir a eficácia de antimicrobianos[38]. Porém, o Octenisept® não apresenta capacidade de dissolução de tecido orgânico[39].

O OCT tem sido sugerido como solução irrigadora e como medicação intracanal em Endodontia com base no seu potente efeito antimicrobiano frente a *S. aureus*[30], *E. faecalis*[29,30,35,40], e *C. albicans*[33,41]. Tandjung et al.[29] mostraram que o Octenisept® em gel foi eficaz na desinfecção de blocos de dentina contaminados com *E. faecalis*; a média de bactérias viáveis diminuiu significantemente de 57,2% para 5,7% depois 10 minutos de aplicação. Após 7 dias, houve o desenvolvimento de bactéria em apenas uma amostra. De Lucena et al.[35] mostraram que o Octenisept® gel empregado como medicação intracanal por 28 dias foi tão eficaz quanto a CHX na eliminação de *E. faecalis* na dentina radicular.

Como solução irrigadora de canais radiculares de dentes de humanos contaminados com *C. albicans*, o Octenisept® apresentou eficácia semelhante ao NaOCl a 2.5 %, ao NaOCl a 5.25 % e a CHX a 2% na eliminação deste micro-organismo[33]. Por meio de teste de contato direto, a solução de Octenisept® foi mais eficaz do que o NaOCl a 5,25% frente a *E. faecalis*, *S. aureus* e *C. albicans* após diferentes intervalos de tempo. O Octenisept® eliminou todos os micro-organismos após 15 segundos de contato direto, enquanto que o NaOCl a 5,25% levou 20 minutos para a eliminação dos micro-organismos[30]. Frente a biofilme de *E. faecalis* formado em blocos de dentina radicular, o Octenisept® apresentou maior atividade antimicrobiana do que a CHX a 2% e menor atividade do que o NaOCl a 5,25%[32]. Além disso, o OCT apresenta efeito residual, sendo que *S. aureus* não foi detectado após 24 horas em epiderme humana reconstruída após sua aplicação de 15 minutos[42].

Em relação aos efeitos citotóxicos, um antisséptico a base de OCT a 0,1%, o Octenidol®, apresentou menor citotoxicidade e induziu menos apoptose do que a CHX a 0,2% em fibroblastos gengivais humanos e células epiteliais nasais[43,44]. Em relação à combinação com outros irrigantes, Octenisept® com o NaOCl gera um precipitado esbranquiçado que foi identificado como fenoxietanol, um componente já presente na formulação original do Octenisept®, e que este precipitado oclui parcialmente os túbulos dentinários[45].

Assim, do ponto de vista antimicrobiano, o Octenisept® pode ser uma solução irrigadora alternativa promissora ao NaOCl e à CHX, mas há necessidade de mais estudos avaliando a sua toxicidade[30,33] e seu efeito sobre a dentina radicular em comparação com esses irrigantes endodônticos.

O hipoclorito de cálcio [$Ca(OCl)_2$] é utilizado para descontaminação de alimentos[46,47], tratamento e purificação de água[48] e branqueamento[49]. Ele está disponível na forma de grânulos e quando preparado em solução aquosa, há liberação de ácido hipocloroso e de hidróxido de cálcio[50]: $Ca(OCl)_2 + 2\ H_2O \rightarrow 2\ HOCl + Ca(OH)_2$. Comparando com o hipoclorito de sódio ($NaOCl + H_2O \rightarrow HOCl + NaOH$) há maior geração de ácido hipocloroso[49], e a presença de Ca^{2+} levaria à produção de duas vezes mais íons hidroxila do que a solução de NaOCl[50]. Além disso, em vez de sódio, é o cálcio que está presente na sua composição, o qual é hipoteticamente mais favorável de ser incorporado na camada híbrida de dentina[51]. O $Ca(OH)_2$ gerado poderia favorecer a atividade antimicrobiana do $Ca(OCl)_2$[49]. Estudo recente mostrou que as soluções de $Ca(OCl)_2$ são altamente alcalinas (pH por volta de 11-12), apresentam maior conteúdo de cloro do que o NaOCl na mesma concentração e maior tensão superficial do que o NaOCl. Em relação ao conteúdo de cloro disponível, a solução de $Ca(OCl)_2$ tende a ser estável até trinta dias quando armazenada a 4 ou a 25°C[52]. A preparação da solução de $Ca(OCl)_2$ pode ser mais precisa do que a preparação de NaOCl porque o pó pode ser pesado e incorporado em água no consultório odontológico, imediatamente antes da utilização clínica. Já a solução de NaOCl é preparada a partir da diluição de uma solução mais concentrada que é instável, o que dificulta a obtenção de uma solução com uma concentração precisa de NaOCl, o que pode interferir com as características desejáveis da solução irrigadora[52].

Em Odontologia, estudos empregando $Ca(OCl)_2$ tem sido realizados para avaliar o seu potencial de dissolução tecidual[50], seu efeito sobre *E. faecalis*[53], sobre a rugosidade da dentina radicular[54] e sobre a microinfiltração de resina composta[51].

Quando empregado como solução irrigadora durante a instrumentação de canais radiculares de dentes extraídos contaminados com *E. faecalis*, o $Ca(OCl)_2$ a 2,5% apresentou eficácia antibacteriana semelhante ao NaOCl a 2,5%, independentemente do uso da irrigação ultrassônica passiva[53]. Na concentração de 5%, o $Ca(OCl)_2$ apresenta a mesma capacidade de dissolução tecidual do que o NaOCl a 5,25%, após 35 e 60 minutos de contato com o tecido[50]. Essa capacidade de dissolução aumenta gradualmente com o tempo e com o aumento da sua concentração[55]. Por outro lado, o $Ca(OCl)_2$, assim como o NaOCl não apresenta capacidade de dissolução de tecido inorgânico e consequentemente não remove a *smear layer* do canal radicular[49].

Em relação aos efeitos sobre a superfície da dentina radicular, o $Ca(OCl)_2$ altera a rugosidade da dentina de forma semelhante ao NaOCl[54]. Quando usado antes de um sistema adesivo à base de acetona, o $Ca(OCl)_2$ não modifica a microinfiltração em relação ao NaOCl. No entanto, o tratamento da dentina com $Ca(OCl)_2$ aumenta a quantidade de íons cálcio e fósforo, o que pode ser benéfico para o processo de mineralização e para a formação de uma fase de fosfato de cálcio amorfo dentro da camada híbrida, já que esses dois elementos representam os principais componentes inorgânicos da dentina[51]. Em relação à toxicidade, Blattes et al.[56] não encontraram diferença entre $Ca(OCl)_2$ e o NaOCl na resposta do tecido subcutâneo de ratos.

Para a seleção da solução irrigadora de canais radiculares deve-se levar em consideração não somente os seus benefícios terapêuticos, mas também os possíveis efeitos citotóxicos, uma vez que ela pode entrar em contato com os tecidos periapicais[57] e a resposta tecidual a esta solução pode influenciar o prognóstico do tratamento endodôntico. Isto torna-se ainda mais crítico quando são empregadas técnicas de regeneração endodôntica em dentes com rizogênese incompleta, que apresentam forame apical amplo, o que permite a solução irrigadora entrar em contato com os tecidos periapicais que são essenciais para que ocorra a regeneração[58]. Também é preciso considerar a capacidade de limpeza do canal radicular pela solução irrigadora. A *smear layer* formada durante o preparo do canal radicular é composta por substâncias orgânicas e inorgânicas e pode conter bactérias e seus subprodutos. Idealmente a *smear layer* deve ser removida[59,60] porque ela pode bloquear o acesso de irrigantes e de medicação no sistema de canais radiculares[61] e influenciar a adaptação[62], união[63] e penetrabilidade dos cimentos endodônticos na dentina radicular[64].

Assim, pesquisas devem ser desenvolvidas na busca de soluções irrigadoras endodônticas que possuam equilíbrio entre as propriedades antimicrobianas e compatibilidade biológica, e que idealmente apresentem capacidade de remoção da *smear layer*.

2 PROPOSIÇÃO

Os objetivos do presente estudo foram:

- Avaliar a citotoxicidade do OCT e do $Ca(OCl)_2$ em comparação com o NaOCl e com a CHX em células L929 e células do ligamento periodontal humano (Publicação 1).
- Avaliar a capacidade de limpeza do OCT como irrigante endodôntico, por meio de microscopia eletrônica de varredura (Publicação 2).

3 PUBLICAÇÕES

3.1 Publicação 1[*]

CYTOTOXICITY OF CALCIUM HYPOCHLORITE AND OCTENIDINE HYDROCHLORIDE IN L929 AND HUMAN PERIODONTAL LIGAMENT CELLS

Abstract

The aim of this study was to assess cytotoxicity of calcium hypochlorite [$Ca(OCl)_2$] and octenidine hydrochloride - OCT (Octenisept®, Schülke & Mayr, Norderstedt, Germany) in L929 and human periodontal ligament (hPDL) cells. The cells were exposed to different doses of different solutions: 2.5% and 5% $Ca(OCl)_2$, 0.1% OCT, 2.5% NaOCl and 2% CHX for 10 minutes. Cell viability was assessed by methyl-thiazol-tetrazolium (MTT) and neutral red (NR) assays, and wound closure was determined by scratch assay. Statistical analysis was performed by two-way ANOVA and Bonferroni tests ($\alpha = 0.05$). The MTT and NR assays revealed that 0.1% OCT was less cytotoxic in hPDL cells ($P < 0.05$), followed by 2% CHX and 2.5% $Ca(OCl)_2$ ($P < 0.05$). There was no significant difference between 2.5% NaOCl and 5% $Ca(OCl)_2$ ($P > 0.05$), but these solutions showed greater cytotoxicity than the others. The result was the same for L929 cells, except that there was no significant difference between 2% CHX and 2.5% $Ca(OCl)_2$ ($P > 0.05$). Scratch assay in L929 and hPDL cells showed that wound closure of 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$ groups was higher than 5% $Ca(OCl)_2$ and 2.5% NaOCl groups at 24 hours ($P < 0.05$). In conclusion, 0.1% OCT had lower cytotoxicity in tested cell lines than CHX, $Ca(OCl)_2$ and NaOCl. $Ca(OCl)_2$ at concentrations of 2.5% and 5% showed cytotoxicity lower than or similar to 2.5% NaOCl, respectively. Therefore, in terms of cytotoxicity, OCT and $Ca(OCl)_2$ have the potential to be used as root canal irrigants.

Keywords: cytotoxicity, calcium hypochlorite, materials testing, octenidine, root canal treatment.

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Introduction

The complex anatomy of the root canal system is a challenge in infection control, especially considering the limitations of biomechanical preparation (De-Deus *et al*. 2010). Current scientific evidence indicates sodium hypochlorite (NaOCl) as the most widely used irrigant solution due to its potent antimicrobial activity (van der Waal *et al*. 2015) and organic dissolution capacity (Dutta & Saunders 2012). However, NaOCl negatively alters the mechanical properties of dentin (Pascon *et al.* 2009), is cytotoxic at high concentrations, and has a pronounced negative effect on the survival and differentiation of stem cells of the apical papilla, factors which may hinder periapical repair and pulpal regeneration (Martin *et al.* 2014). CHX is a potent antiseptic used in endodontic treatment due to its antimicrobial efficacy (van der Waal *et al*. 2015) and substantivity (Carrilho *et al*. 2010). However, CHX cannot dissolve organic tissues (Okino *et al*. 2004). Currently, there is no root canal irrigant considered ideal (Haapasalo *et al.* 2014), and alternative solutions continue to be studied.

Calcium hypochlorite [$Ca(OCl)_2$] is used for treatment and purification of water (Patil *et al*. 2013), and has been studied as a root canal irrigant (de Almeida *et al*. 2014, Blattes *et al*. 2017). It has tissue dissolution capacity (Dutta & Saunders 2012) and higher chlorine content than NaOCl at the same concentration (Leonardo *et al*. 2016). The preparation of a $Ca(OCl)_2$ solution may be more accurate than that of NaOCl, because $Ca(OCl)_2$ powder can be weighed and incorporated into water prior to use. On the other hand, a NaOCl solution is prepared by diluting a more concentrated and therefore unstable solution, thus making it difficult to obtain an accurate concentration of NaOCl (Leonardo *et al*. 2016). When used as an irrigant solution during biomechanical preparation of *Enterococcus faecalis*-infected teeth, 2.5% $Ca(OCl)_2$ showed antibacterial efficacy similar to 2.5% NaOCl (de Almeida *et al*. 2014). Regarding cytotoxic effects, Blattes *et al*. (2017) found no difference between $Ca(OCl)_2$ and NaOCl in 3T3 embryonic mouse fibroblast cells.

Octenisept® - OCT (Schülke & Mayr, Norderstedt, Germany) contains 0.1% octenidine hydrochloride (an antimicrobial agent) and 2% phenoxyethanol, a derivative of ethanol, which serves as a preservative (Tandjung *et al*. 2007), and which synergistically improves the antibacterial activity of octenidine (Bukhary & Balto 2017). OCT is used primarily for antisepsis of burns and wounds, and as a mouthwash (Tandjung *et al*. 2007, Tirali *et al*. 2009). As a root canal irrigant, OCT has showed similar efficacy to 2.5% and 5.25% NaOCl, and to 2% CHX against *Candida albicans*

(Eldeniz *et al*. 2015) and *E. faecalis* (Guneser *et al*. 2016). In a direct contact test, OCT performed better than 5.25% NaOCl against *E. faecalis*, *Staphylococcus aureus* and *C. albicans* (Tirali *et al*. 2009). In root dentin blocks, OCT showed higher antibacterial activity against *E. faecalis* biofilm than 2% CHX, but lower than 5.25% NaOCl (Bukhary & Balto 2017). In addition, OCT has a residual effect, that is, a 15-minute application of OCT resulted in non-detection of *S. aureus* after 24 hours in the reconstructed human epidermis (Müller *et al*. 2014). Regarding cytotoxic effects, another 0.1% octenidine hydrochloride-based antiseptic, Octenidol®, has presented cytotoxicity lower than 0.2% CHX in human gingival fibroblasts and nasal epithelial cells (Schmidt *et al*. 2016).

Therefore, OCT and $Ca(OCl)_2$ have the potential to be used as alternative root canal irrigants to NaOCl and CHX. However, further studies comparing the cytotoxicity of OCT and $Ca(OCl)_2$ with that of other root canal irrigants in different cell lines are needed to indicate these solutions for endodontic treatment. The aim of this study was to assess the cytotoxicity of OCT and $Ca(OCl)_2$, in comparison with NaOCl and CHX, in human periodontal ligament (hPDL) cells and L929 fibroblasts. The null hypothesis was that there would be no difference in the cytotoxicity of the tested solutions.

Materials and methods (extended version in appendix A)

Preparation of irrigant solutions

The solutions evaluated were 2.5% and 5% $Ca(OCl)_2$ (Sigma-Aldrich, St. Louis, MO, USA), 0.1% OCT (Octenisept®, Schulke & Mayr), 2.5% NaOCl (AraQuímica, Araraquara, SP, Brazil) and 2% CHX (Reactive Manipulation Pharmacy, Araraquara, SP, Brazil). $Ca(OCl)_2$ solution was prepared immediately prior to use by diluting $Ca(OCl)_2$ powder in distilled water, and 2.5% NaOCl was prepared by diluting 9% NaOCl solution in distilled water. The available chlorine content in NaOCl and in $Ca(OCl)_2$ solutions was determined by the physicochemical spectrophotometric method (Rice *et al.* 2012). The $Ca(OCl)_2$ concentrations of 2.5% and 5%, as well as 0.1% OCT, 2.5% NaOCl and 2% CHX, were considered grade 1 dilutions, and were serially diluted in saline solution (0.9% sodium chloride) using a 1.5 dilution factor (Viola *et al.* 2017). The cells were incubated with solutions in the following dilutions: 1/111, 1/166, 1/250, 1/375, 1/562, 1/844, 1/1266 and 1/1898, which corresponded to doses/concentrations of 0.9%, 0.6%, 0.4%, 0.26%, 0.18%, 0.12%, 0.08% and 0.05%, respectively.

Cell culture and treatment protocol with the irrigant solutions

Permanent cell lines of L929 mouse fibroblasts (American Type Culture Collection) and human hPDL cells were used. All procedures conformed to the applicable ethical guidelines and regulations of the dental school’s Research Ethics Committee, which approved the project (no. 57134916.3.0000.5416). Human third molars with no evidence of carious lesions or periodontal disease were obtained from healthy patients aged 16-25 year, who were being treated at the dental school’s surgery clinic. After extraction, the teeth were immediately stored in Dulbecco’s Modified Eagle’s Medium - DMEM (Sigma-Aldrich, St. Louis, MO, USA). The periodontal ligament was removed from the middle third of the root surface with a #15 scalpel blade, and then fragmented and cultured in 100-mm culture dishes using the explant technique (Lin *et al*. 2004). hPDL cells from the 3rd through 6th passages were used, and assays were performed in triplicate, using cells from 3 donors.

Both hPDL and L929 cells were cultured with DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco / Invitrogen, Waltham, MS, USA), 1% penicillin and streptomycin (Sigma-Aldrich) (100.00U / mL penicillin, 100.00 mg / mL streptomycin), and were incubated at 37°C in an atmosphere containing 5% CO_2 and 95% humidity.

The assays were performed by culturing cells in 24- or 96-well culture plates (Corning Inc., Corning, NY, USA), containing DMEM with 10% FBS, and incubating them for 24 hours in order for them to adhere to the wells. Then, the culture medium was removed, and the cells were incubated with different doses of the solutions, for a period of 10 minutes (Viola *et al*. 2017). The solutions were then removed, and the cells were incubated with culture medium containing 10% FBS for 4 hours (Giannelli *et al*. 2008, Viola *et al*. 2017). Saline and DMEM were used as controls.

Cell viability evaluation by methyl-thiazol-tetrazolium (MTT) and neutral red (NR) assays

The hPDL ($8x10^4$ cells/mL) and L929 ($6.5x10^4$ cells/mL) cells were cultured in 96-well plates (Corning). The cell treatment protocol was performed with the tested solutions at doses of 0.05–0.9% and with the controls, after which the culture medium was removed and the MTT and NR assays were performed.

For the MTT assay, 100 µl of the 0.5 mg/ml MTT solution (Sigma-Aldrich) was added, and the cells were incubated for 3 hours at 37°C, in an atmosphere containing

5% CO_2 and 95% relative humidity. Then, 100 µL of acidified isopropyl alcohol (HCl: isopropyl alcohol, 0.04N) was added to the extract to solubilize the formazan crystals. The optical densities of the solutions were measured by spectrophotometer with a 570 nm wavelength filter (ThermoPlate, Nanshan District, Shenzhen, China). The absorbance readings were normalized with cells exposed to the saline, and represented succinate dehydrogenase activity (cell metabolism).

For the NR assay, 100 µL of 0.05 mg/mL NR solution (Sigma-Aldrich) was added, and the cells were incubated at 37°C, in an atmosphere containing 5% CO_2 and 95% relative humidity for 3 hours. Then, the solution was discarded and 100 µL of 1% acetic acid solution in 50% ethanol was added to each well. The optical densities of the solutions were measured by spectrophotometer with 570 nm wavelength filters (ThermoPlate). The absorbance readings were normalized with cells exposed to saline solution, and represented the ability to incorporate the dye into lysosomes of viable cells. The assays were performed in triplicate and repeated at three different times.

Wound closure evaluation by scratch assay

As previously described (Liang *et al.* 2007), an established *in vitro* wound healing model was used to analyze the effects of tested solutions on the wound closure. The hPDL ($5x10^5$ cells/mL) and L929 ($2.5x10^5$ cells/mL) cells were cultivated in 24-well culture plates (Corning). Then, the cells were removed with a P200 tip (TPP, Techno Plastic Products, Trasadigen, Switzerland), by creating an artificial gap, or so-called “scratch” or “wound,” through the center of each well. Immediately afterwards, the cells were washed twice with phosphate-buffered saline (PBS) and exposed to the treatment protocol with the tested solutions at a dose of 0.05% and the controls. To determine the cell growth area, the wells were photographed at 0, 8, 16 and 24 hours by EVOS F1 microscope (AMC, Bothell, WA, USA), and the images were analyzed by two blinded and calibrated examiners, using 145 ImageJ software (National Institutes of Health, NIH, Bethesda, Maryland, USA). The experiments were performed in quadruplicate and repeated at two different times. Eight different fields per well were photographed and analyzed.

Statistical analysis

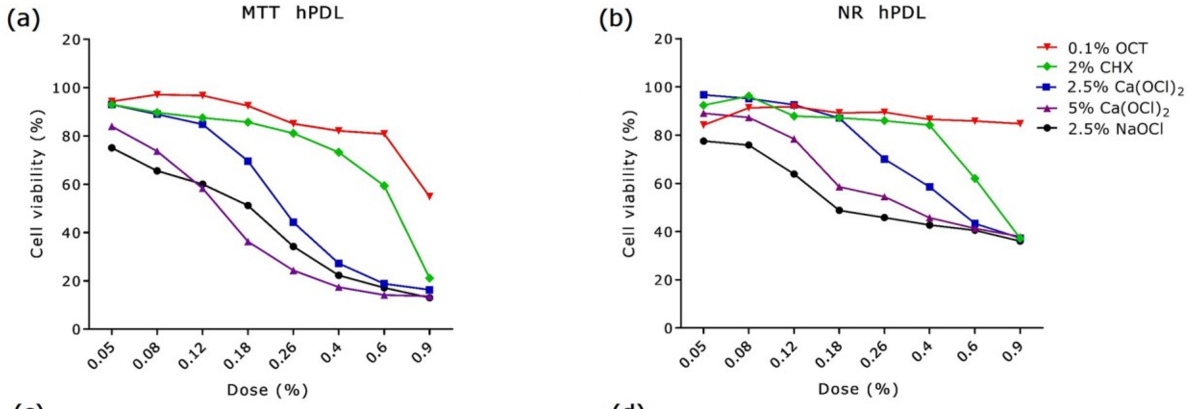
The data were analyzed with Graph Pad Prism 5 statistical software (GraphPad Software, La Jolla, CA, USA), by using two-way ANOVA and the Bonferroni post-test ($\alpha = 0.05$).

Results

Cell viability evaluation by MTT and NR assays

Cell viability is shown in Figures 1 and 2. All the solutions had a dose-dependent effect on cell viability; the higher the dose, the greater the cytotoxicity. The MTT and NR assays on hPDL cells revealed that 0.1% OCT was the least cytotoxic ($P < 0.05$), followed by 2% CHX and 2.5% $Ca(OCl)_2$ ($P < 0.05$). There was no significant difference between 2.5% NaOCl and 5% $Ca(OCl)_2$ ($P > 0.05$), but these solutions had greater cytotoxicity than the others. The result was the same for the L929 cells, except that there was no significant difference between 2% CHX and 2.5% $Ca(OCl)_2$ ($P > 0.05$). There was also no significant difference ($P > 0.05$) in the response of the cell lines to saline and DMEM (controls).

A comparison of the response of both cell lines in the MTT assay showed that 0.1% OCT, 2.5% and 5% $Ca(OCl)_2$ were more cytotoxic to hPDL than L929 cells ($P < 0.05$), and that 2% CHX was more cytotoxic to L929 than hPDL cells ($P < 0.05$). There was no significant difference between the response of cell lines exposed to 2.5% NaOCl ($P > 0.05$).



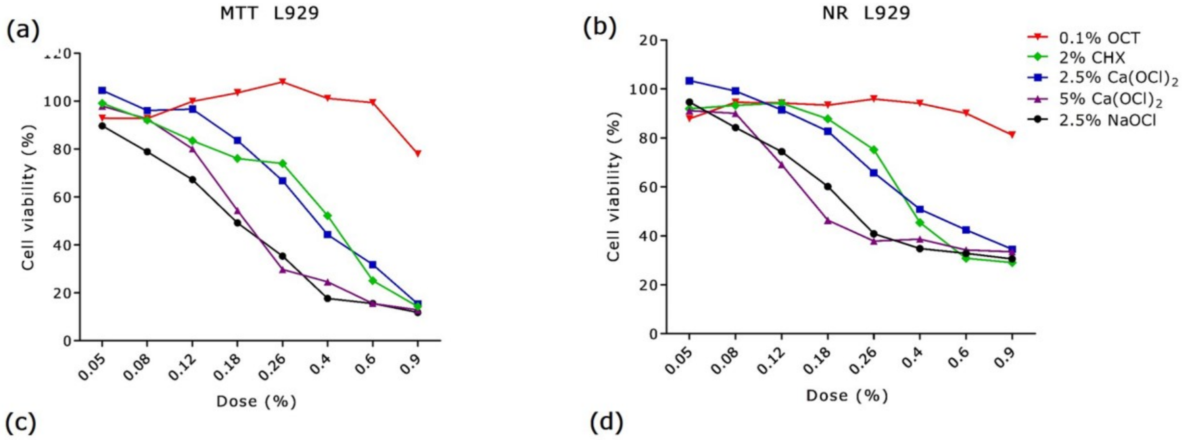
(c)

		0.05	0.08	0.12	0.18	0.26	0.4	0.6	0.9
🟥	0.1% OCT	a	c	b	c	c	b	b	b
🟩	2% CHX	a	bc	b	bc	c	b	c	a
🟦	2.5% $Ca(OCl)_2$	a	bc	b	b	b	a	a	a
🟪	5% $Ca(OCl)_2$	a	ab	a	a	a	a	a	a
⬛	2.5% NaOCl	a	a	a	a	ab	a	a	a

(d)

		0.05	0.08	0.12	0.18	0.26	0.4	0.6	0.9
🟥	0.1% OCT	ab	b	b	b	c	c	b	b
🟩	2% CHX	bc	b	b	b	c	c	c	a
🟦	2.5% $Ca(OCl)_2$	bc	b	b	b	b	b	a	a
🟪	5% $Ca(OCl)_2$	ab	ab	ab	a	a	a	a	a
⬛	2.5% NaOCl	a	a	a	a	a	a	a	a

Figure 1. Viability of human periodontal ligament (hPDL) cells after exposure to solutions tested at different doses by (a) methyl-thiazol-tetrazolium (MTT) and (b) neutral red (NR) assays. Statistical comparison of MTT (c) and NR (d) results: different letters in columns indicate statistically significant differences among the solutions.



(c)

		0.05	0.08	0.12	0.18	0.26	0.4	0.6	0.9
🟥	0.1% OCT	a	ab	c	c	c	c	c	b
🟩	2% CHX	a	ab	bc	b	b	b	ab	a
🟦	2.5% $Ca(OCl)_2$	a	b	c	b	b	b	b	a
🟪	5% $Ca(OCl)_2$	a	ab	ab	a	a	a	a	a
⬛	2.5% NaOCl	a	a	a	a	a	a	a	a

(d)

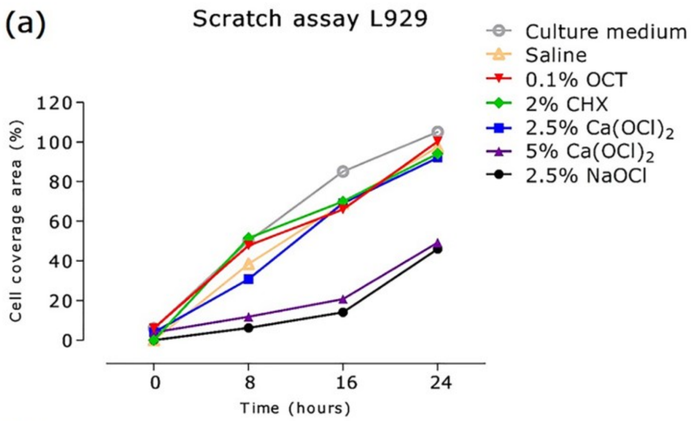
		0.05	0.08	0.12	0.18	0.26	0.4	0.6	0.9
🟥	0.1% OCT	b	ab	b	b	c	c	c	b
🟩	2% CHX	b	ab	b	b	b	bd	a	a
🟦	2.5% $Ca(OCl)_2$	a	b	b	b	b	b	b	a
🟪	5% $Ca(OCl)_2$	b	ab	a	c	a	ad	ab	a
⬛	2.5% NaOCl	ab	a	a	a	a	a	ab	a

Figure 2. Viability of L929 fibroblasts after exposure to solutions tested at different doses by (a) methyl-thiazol-tetrazolium (MTT) and (b) neutral red (NR) assays. Statistical comparison of MTT (c) and NR (d) results: different letters in columns indicate statistically significant differences among the solutions.

Wound closure evaluation by scratch assay

In regard to the L929 cells (Figure 3), at 8 hours, the controls (culture medium and saline), as well as 0.1% OCT and 2% CHX showed higher wound closure than 2.5% $Ca(OCl)_2$ ($P < 0.05$). Lower wound closure was observed for 5% $Ca(OCl)_2$ and 2.5% NaOCl ($P < 0.05$). At 16 and 24 hours, there was no significant difference among the controls, 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$ ($P > 0.05$). These groups had higher wound closure than the 5% $Ca(OCl)_2$ and 2.5% NaOCl groups ($P < 0.05$).

A similar pattern was observed in hPDL cells (Figure 4), but at 16 hours, the controls showed higher wound closure than the other solutions ($P < 0.05$). At 24 hours, no differences were found among the controls, 2% CHX, 0.1% OCT and 2.5% $Ca(OCl)_2$ ($P > 0.05$), showing that these solutions had higher wound closure than 5% $Ca(OCl)_2$ and 2.5% NaOCl ($P < 0.05$).



(b)

		0 h	8 h	16 h	24 h
⬜	Culture medium	a	a	a	a
🟧	Saline	a	a	a	a
🟥	0.1% OCT	a	a	a	a
🟩	2% CHX	a	a	a	a
🟦	2.5% $Ca(OCl)_2$	a	c	a	a
🟪	5% $Ca(OCl)_2$	a	b	b	b
⬛	2.5% NaOCl	a	b	b	b

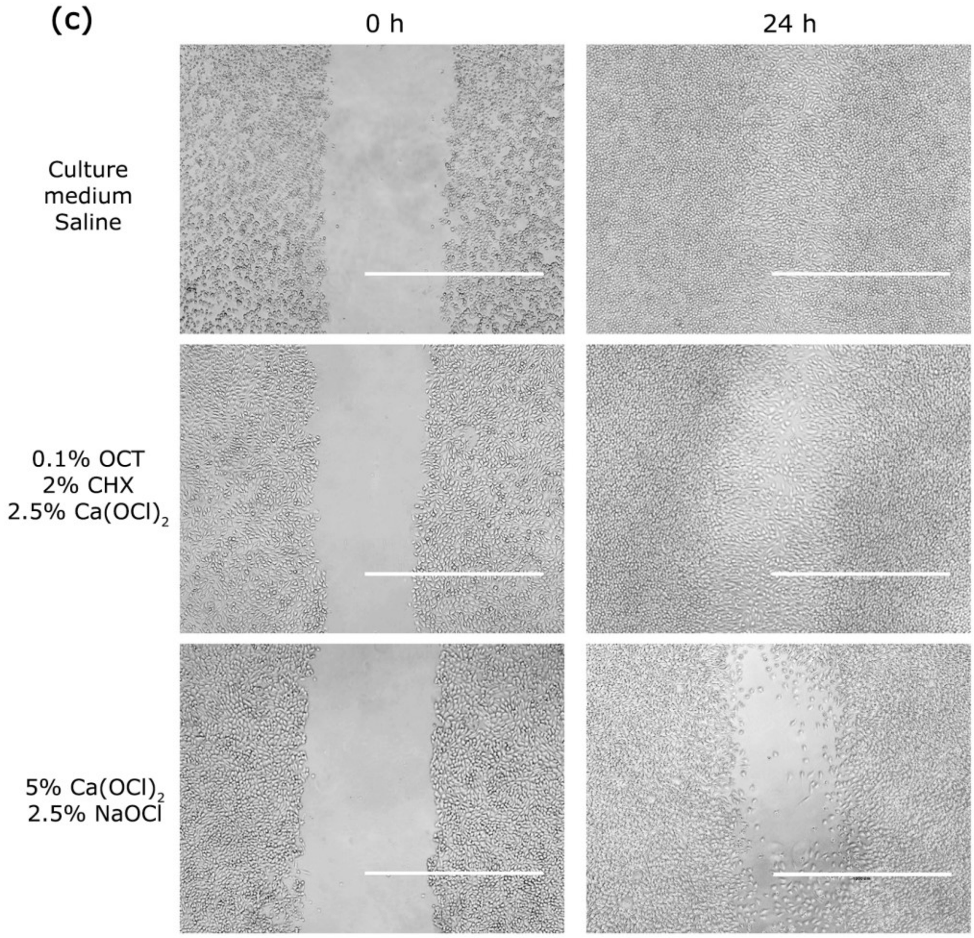
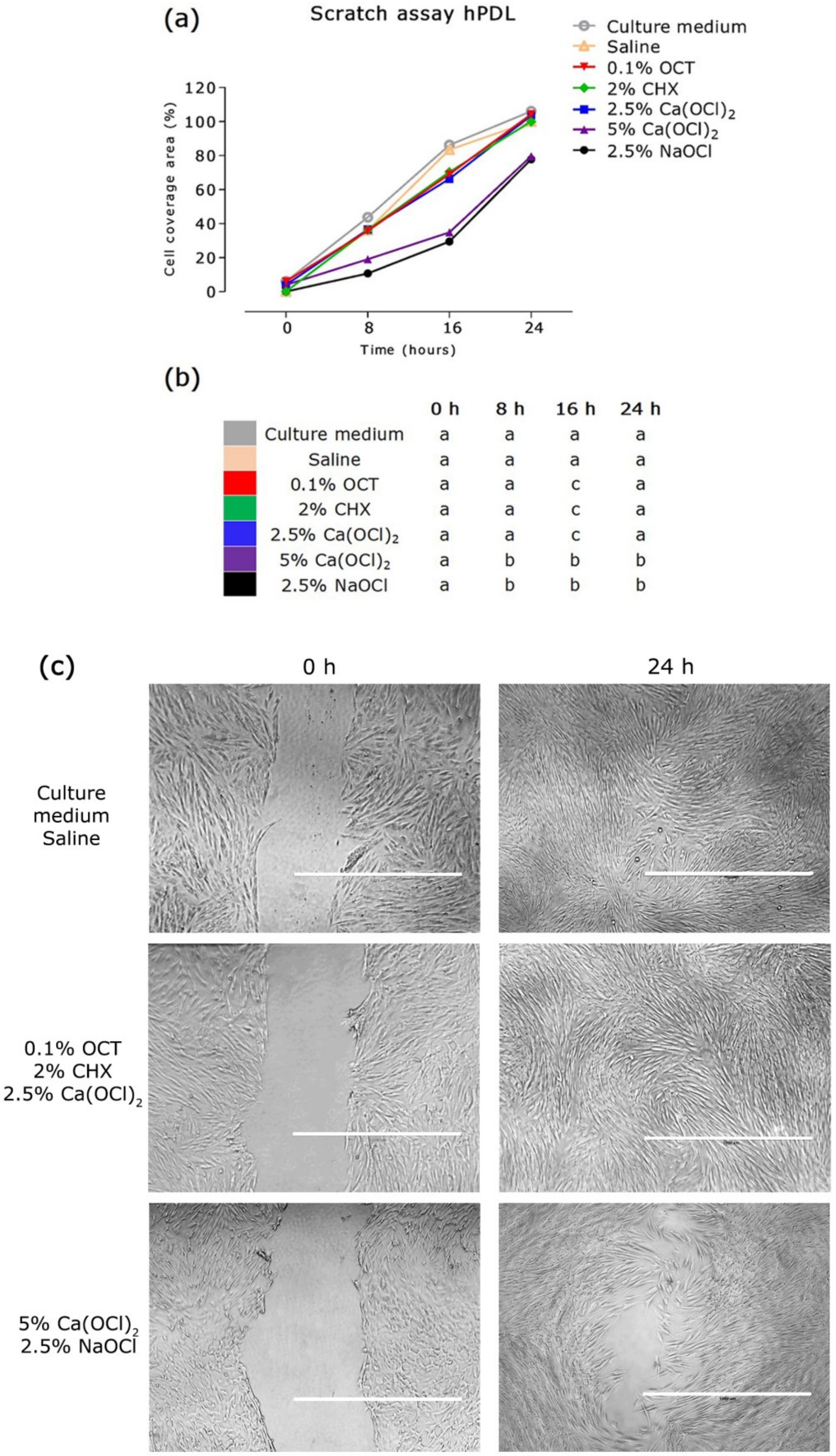


Figure 3. Wound closure (in percentage of cell coverage area) in L929 fibroblasts after exposure to tested solutions at 0.05% for 10 minutes (a). Statistical comparison of results: different letters in columns indicate significant differences among the solutions (b). Representative images of cell coverage at 0 and 24 h. Bar = 1000 µm (c).



	0 h	8 h	16 h	24 h
Culture medium	a	a	a	a
Saline	a	a	a	a
0.1% OCT	a	a	c	a
2% CHX	a	a	c	a
2.5% $Ca(OCl)_2$	a	a	c	a
5% $Ca(OCl)_2$	a	b	b	b
2.5% NaOCl	a	b	b	b

Figure 4. Wound closure (in percentage of cell coverage area) in human periodontal ligament (hPDL) cells after exposure to solutions tested at 0.05% for 10 minutes (a). Statistical comparison of results: different letters in columns indicate significant differences among the solutions (b). Representative images of cell coverage at 0 and 24 h. Bar = 1000 µm (c).

Discussion

The aim of this study was to assess the cytotoxicity of OCT and $Ca(OCl)_2$ in different cell lines, compared with CHX and NaOCl. The null hypothesis was rejected because there were differences in the cytotoxicity of the solutions.

L929 fibroblasts were used because they are recommended by ISO 10993 (ISO 10993-5, 2009), have good reproducibility (Schmalz 1994), and are frequently used in cytotoxicity assays of dental materials (Andolfatto *et al*. 2017, Viola *et al*. 2017). However, permanent cell lines may have alterations in biological processes, components and cellular functions (Hou *et al*. 2013), factors which could influence the results of cytotoxicity assays (Thonemann *et al*. 2002). hPDL cells were used because they are affected by irrigant solutions that could extrude to the periapical region, and the response of these cells to irrigant solutions may influence the prognosis of the endodontic therapy. Primary cells have specific metabolic potential, almost like target cells of dental materials in tissues (Schmalz 1994) – although laborious and time-consuming to isolate – exhibit low cell profitability and have a limited number of passages (Thonemann *et al*. 2002, Hou *et al*. 2013).

The tested solutions were diluted in saline solution instead of culture medium or PBS, because the latter options contain buffering substances that can alter the pH of the solutions, and thus also alter the clinical situation of use (Viola *et al*. 2017). Both MTT and NR assays were performed, because it is recommended to perform more than one assay for *in vitro* studies, to obtain a more reliable assessment of cytotoxicity (Fotakis & Timbell 2006). The MTT assay is based on the ability of viable cells to metabolically reduce soluble yellow MTT salt to insoluble blue-violet formazan crystals, insoluble in aqueous solutions, whose absorbance after dilution in alcohol is proportional to the number of viable cells (ISO 10993-5, 2009). NR is a viability assay based on the ability of viable cells to incorporate this dye into their lysosomes, where it accumulates when the cell membrane is intact (Repetto *et al*. 2008).

MTT and NR assays revealed that all the solutions, in both cell lines, had an effect on cell viability in a dose-dependent manner. OCT was less cytotoxic in comparison with the other solutions. Schmidt *et al*. (2016) demonstrated that, in relation to human gingival fibroblasts and nasal epithelial cells, another 0.1% octenidine hydrochloride-based antiseptic, Octenidol®, presented lower cytotoxicity than 0.2% CHX.

$Ca(OCl)_2$ at a concentration of 2.5% was less cytotoxic than 2.5% NaOCl. Blattes *et al*. (2017) observed no difference in cytotoxicity between $Ca(OCl)_2$ and NaOCl at the same concentration. This discrepancy could be attributed to methodology, since Blattes *et al*. (2017) evaluated the viability in fibroblasts exposed to solutions for 24 hours. In the present study, the cells were exposed to solutions for ten minutes, because longer periods of exposure, such as 24 hours, allow cells to recover from reversible toxic effects, considering that hypochlorite solutions present rapid breakdown kinetics. The maximum chlorine is released within the first 4 h, and thereafter the real hypochlorous acid (HOCl) concentration decreases progressively and significantly according to the exposure time (Hidalgo *et al*. 2002).

The comparison of two cell line responses to the solutions showed that OCT and $Ca(OCl)_2$ were more toxic to hPDL than L929 cells ($P < 0.05$), and CHX was more toxic to L929 than hPDL cells ($P < 0.05$). This can occur because different cells can be affected in varying degrees of severity, depending on the target mechanism for the cytotoxic action of the substance (Al-Nazhan & Spangberg 1990).

The results of the 2.5% NaOCl group corroborate those of the study by Viola *et al*. (2017), which showed the same pattern of cellular metabolism lost in the MTT assay, when L929 cells were exposed to 2.5% NaOCl, using the same protocol as the present study. According to these authors, the mechanism of cytotoxicity of NaOCl includes a decrease in cellular metabolism, cytoskeletal deconstruction, accumulation of proteins in the rough endoplasmic reticulum and induction of cell death predominantly by necrosis. In hPDL and L929 cells, 2% CHX showed lower cytotoxicity than 2.5% NaOCl, which corroborates the findings of a recent study showing that 2% CHX had less cytotoxic potential than 2.5% NaOCl in mononuclear blood cells (Botton *et al*. 2016).

The scratch assay is based on the creation of an artificial gap in the cell monolayer and in the capture of images at certain periods, and serves to analyze, to some extent, cell migration and proliferation to close the wound (Liang *et al.* 2007, Nokhbehsaim *et al*. 2014). In L929 and hPDL cells, the wound closure in the 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$ groups was higher than that of the 5% $Ca(OCl)_2$ and the 2.5% NaOCl groups at 24 hours. These results corroborate those of Blattes *et al*. (2017), who showed higher wound closure in the $Ca(OCl)_2$ group than in the NaOCl group, and also those of Jenull *et al*. (2015), who observed that the wound in fibroblasts exposed to OCT for 2 minutes had closed completely within 24 hours, as was shown in the present study.

In conclusion, 0.1% OCT had lower cytotoxicity than 2% CHX, 2.5% and 5% $Ca(OCl)_2$, and 2.5% NaOCl. $Ca(OCl)_2$ at concentrations of 2.5% and 5% showed cytotoxicity lower than or similar to 2.5% NaOCl, respectively. Therefore, in terms of cytotoxicity, OCT and $Ca(OCl)_2$ have the potential to be used as root canal irrigants *in vivo*.

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Conflict of interest

The authors hereby state explicitly that there is no conflict of interests in connection with this article.

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3.2 Publicação 2*

CLEANING CAPACITY OF OCTENIDINE AS ROOT CANAL IRRIGANT: A SCANNING ELECTRON MICROSCOPY STUDY

ABSTRACT

The aim of this study was to assess the cleaning capacity of the octenidine hydrochloride (OCT) used as root canal irrigant by scanning electron microscopy (SEM) analysis. Fifty human unirradicular extracted teeth were randomly distributed in 5 groups (n = 10) according to irrigant solutions which were used during root canal preparation: G1, 0.1% OCT; G2, 2% chlorhexidine (CHX); G3, 2.5% sodium hypochlorite (NaOCl); G4, OCT + 17% ethylenediaminetetraacetic acid (EDTA) and G5, 2.5% NaOCl + 17% EDTA. All specimens were instrumented with ProTaper system up to F4. Teeth were sectioned and prepared for SEM analysis. The smear layer was evaluated using a 5-score system and the data were analyzed by Kruskal-Wallis and Dunn ($\alpha = 0.05$). In all root canal thirds there was no significant difference between OCT, CHX and NaOCl groups ($p > .05$), and these groups showed higher smear layer values than NaOCl + EDTA and OCT + EDTA groups ($p < .05$). There was no significant difference between NaOCl + EDTA and OCT + EDTA groups ($p > .05$). It was concluded that OCT used as a single root canal irrigant presented poor cleaning capacity and could be used in association with a final irrigation with EDTA to obtain smear layer removal.

Key Words: chlorhexidine, octenidine hydrochloride, smear layer, sodium hypochlorite.

Research Highlights: Octenidine could be used as root canal irrigant. However, it should be used with a final irrigation with EDTA in order to improve root canal cleaning.

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A autorização para o uso do artigo nesta dissertação encontra-se no Anexo B.

INTRODUCTION

The success of root canal treatment is dependent on multiple factors (Ørstavik, Qvist & Stoltze, 2004), and root canal irrigants has a high influence (Haapasalo, Shen, Wang & Gao, 2014). Several irrigants have been used in endodontics; among them sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most commonly used (Zehnder, 2006).

NaOCl is widely recommended due to its organic tissue dissolution capacity and antimicrobial activity (Torabinejad, Handysides, Khademi & Bakland, 2002). However, NaOCl is highly irritating when in contact with periapical tissues (Farook et al., 2014), negatively alters the mechanical properties of dentin, such as microhardness (Aslantas, Buzoglu, Altundasar & Serper, 2014), modulus of elasticity, and flexural strength (Marending et al., 2007), and may reduce the bond strength of root canal sealers (Neelakantan, Sharma, Shemesh & Wesselink, 2015).

CHX has been recommended as root canal irrigant based on its antibacterial effects (Zandi et al., 2016) and substantivity, which allows prolonged residual effect (Souza et al., 2012). However, CHX does not dissolve organic tissues (Okino, Siqueira, Santos, Bombana & Figueiredo, 2004). In relation to biocompatibility, there is no consensus on the effect of CHX in connective tissue. Some studies shows that it induces tissue necrosis and intense inflammatory response (Faria et al., 2007; Pereira et al., 2012), while others show good periapical regeneration when CHX is used as root canal irrigant or intracanal dressing (Tanomaru Filho, Leonardo & da Silva, 2002; Dammaschke, Schneider, Stratmann, Yoo & Schäfer, 2005).

Alternative irrigants, among them octenidine hydrochloride (OCT), have been studied to improve the disinfection of root canal system (Tirali, Turan, Akal & Karahan, 2009; Bukhary & Balto, 2017). Octenisept (Schülke & Mayr, Nordersdedt, Germany) is an antimicrobial agent used for burns antisepsis, surgical wounds of the skin and mouth rinse. It is composed of 0.1% OCT and phenoxyethanol, an ethanol derivative, which serves as a preservative (Tandjung et al., 2007; Tirali et al., 2009). OCT interferes with cell wall and membrane of microorganisms, showing bactericidal and fungicidal activity (Tandjung et al., 2007). It has shown potent antimicrobial effect against *Staphylococcus aureus* (Tirali et al., 2009), *Enterococcus faecalis* (Tandjung et al., 2007, Tirali et al., 2009; de Lucena et al., 2013; Bukhary & Balto, 2017) and *Candida albicans* (Eldeniz, Guneser & Akbulut, 2015; Tirali, Bodur, Sipahi & Sungurtekin, 2013). Furthermore, OCT inhibits biofilm formation and disrupts fully formed biofilm even in presence of serum protein (Amalaradjou & Venkitanarayanan, 2014). OCT at concentrations of 0.03%, 0.05% and 0.10% has similar effect in comparison to 5.25% NaOCl against *C. albicans* and *E. faecalis*, and higher effect in comparison to 0.12% and 2% CHX (Tirali et al., 2013). Additionally, OCT has greater antimicrobial activity against *E. faecalis* biofilms compared with CHX (Bukhary & Balto, 2017), and presents faster ability to produce intratubular disinfection than NaOCl and CHX (Tirali, Bodur & Ece, 2012). Therefore, from an antimicrobial view, OCT may be an alternative root canal irrigant. However, there are no studies in the literature evaluating the cleaning capacity of OCT used as root canal irrigant.

The aim of this study was to assess, in vitro, the cleaning capacity of OCT as root canal irrigant by scanning electron microscopic (SEM) analysis. The null hypothesis was that there would be no difference on the cleaning capacity of OCT, CHX, NaOCl, NaOCl associated with EDTA or OCT associated with EDTA.

MATERIALS AND METHODS (extended version in appendix A)

Specimen preparation

All procedures conformed to the applicable ethical guidelines and regulations of Faculty's Research Ethics Committee, which approved the project (number 65131917.1.0000.5416; attachment C). Fifty unirradicular human mandibular premolars were digitally radiographed in both buccolingual and mesiodistal directions in order to confirm a single root canal and similar morphology. Exclusion criteria included complex root canal anatomy, presence of calcifications, open apex, apical curvatures, external or internal root resorption, or canals that allowed the introduction of an instrument exceeding ISO size 20 to the apical foramen. The selected teeth were stored in 0.1% thymol until use. The teeth were decorated 16 mm from the anatomic apex. The working length of each root canal was established 1 mm from the apical foramen. The foraminal opening was sealed with resin composite to prevent irrigant extrusion from the apical foramen. The roots were randomly distributed into five groups (n = 10 per group) according to the irrigation protocol used for root canal preparation: G1, 0.1% OCT (Octenisept, Schülke & Mayr); G2, 2% CHX (Arte & Ciência Farmácia de Manipulação, Araraquara, SP, Brazil); G3, 2,5% NaOCl (Asfer Indústria Química Ltda., São Caetano do Sul, SP, Brazil); G4, 0.1% OCT during instrumentation followed by final irrigation with 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) and G5, 2.5% NaOCl during instrumentation, followed by final irrigation with 17% EDTA. Root canal preparation was performed by the same operator using ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 file at 300 rpm speed and 1.5 – 3 Ncm torque by using an electric engine (X-Smart Plus; Dentsply Maillefer, Ballaigues, Switzerland). Between each file, the irrigation were performed with 2 mL of the tested irrigants for 1 minute. In the groups that included 17% EDTA, the specimens were irrigated with 3 mL of this solution for 3 minutes. At the end of procedure, all groups were irrigated with 3 mL of distilled water for 2 minutes and dried with absorbent paper points. Root canal irrigants were placed in 5-mL syringes (Ultradent Products, South Jordan, UT, USA) with an endodontic 30G side-vented needle (NaviTip, Ultradent Products, South Jordan, UT, USA), which was placed inside the root canal 2 mm short of the working length with back-and-forth movements of 2-3 mm. Simultaneously, suction was accomplished by using a metal cannula.

Scanning electron microscopy (SEM) evaluation

After root canal preparation, parallel grooves were made along buccal and lingual surfaces of teeth with a diamond disc without water cooling, preserving the inner shelf of dentin surrounding the canal. Then, all roots were sectioned by using chisel and hammer along the longitudinal axis, and the specimens were dehydrated, coated with gold and examined under a scanning electron microscope (EVO 50, Zeiss, Cambridge, UK) at 20 KV, in accordance with the methodology of Faria et al. (2014). Initially the root canal walls were visualized at 500X magnification in different fields in the apical, middle and cervical thirds. Three representative SEM photomicrographs were taken at 2000X magnification in apical, middle and cervical thirds of each specimen for analysis of the smear layer. The smear layer was scored using the system proposed by Hülsmann, Rümmelin & Schäfers (1997): 1, no smear layer with dentinal tubule open; 2, small amount of smear layer with some dentinal tubule open; 3, homogenous smear layer covering the root canal wall with only few dentinal tubule open; 4, complete root canal wall covered by a homogenous smear layer with no open dentinal tubule and 5, heavy non homogenous smear layer covering the complete root canal wall. All samples were independently analyzed in a blind manner by two calibrated examiners (Kappa > 0.9). Values were compared, and when there was a difference, the examiners jointly inspected the sample until they reached agreement on scoring. Data were analyzed by Kruskal-Wallis and Dunn tests at 5% significance level, using the Graph Pad Prism 5 software (Graph Pad Software In., San Diego, CA, USA).

RESULTS

Figure 1 shows the distribution of scores at the cervical, middle and apical levels. In all thirds, there was no significant difference among NaOCl, OCT and CHX groups (*p* > .05), and these groups showed higher smear layer values than NaOCl + EDTA and OCT + EDTA groups. There was no significant difference between NaOCl + EDTA and OCT + EDTA groups (*p* > .05). OCT, NaOCl + EDTA, and OCT + EDTA groups had lower smear layer values in the cervical than apical thirds (*p* < .05). NaOCl group had no significant difference in cervical, middle and apical thirds (*p* > .05). Figures 2 and 3 show representative SEM images of the dentin aspect in different root thirds of the groups.

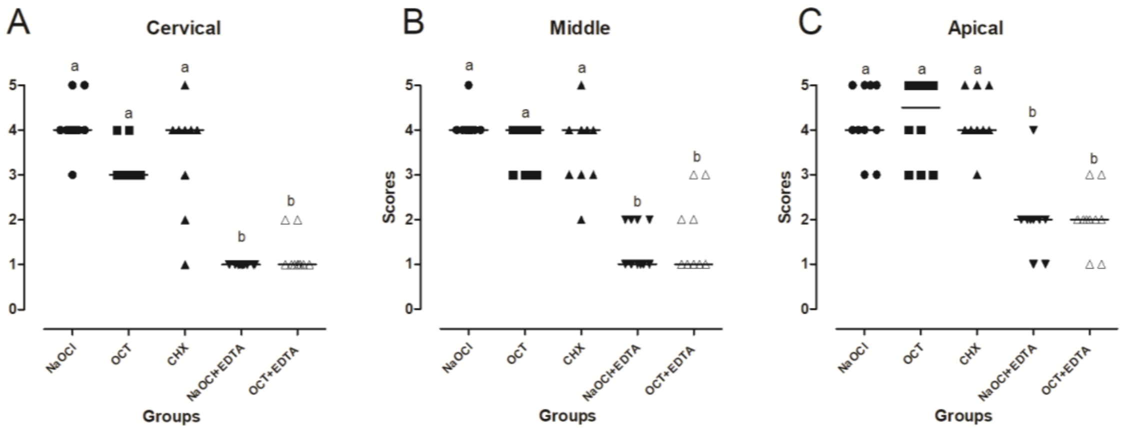

Figure 1. Comparison of smear layer scores at cervical (A), middle (B) and apical (C) thirds. Different letters indicate statistically significant differences.

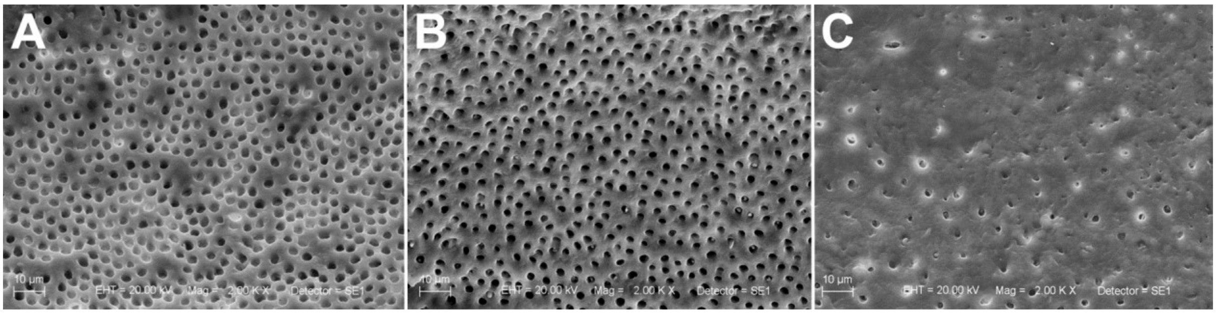

Figure 2. Representative SEM images of NaOCl + EDTA and OCT + EDTA groups at cervical (A), middle (B) and apical (C) thirds. SEM images of cervical and middle thirds represent score 1 and SEM image of apical third represents score 2. Bar = 10 µm.

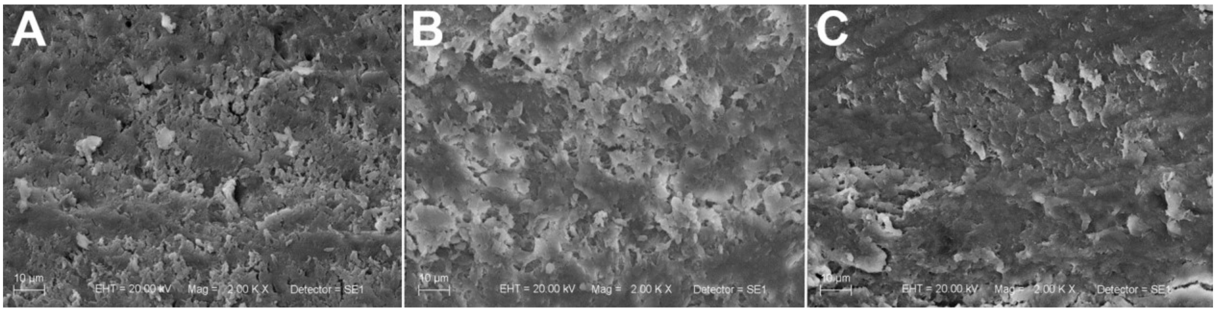

Figure 3. Representative SEM images of OCT, CHX and NaOCl groups at cervical (A), middle (B) and apical (C) thirds. SEM images of all thirds represent score 4. Bar = 10 µm.

DISCUSSION

This study aimed to evaluate the cleaning capacity of OCT as root canal irrigant in comparison with NaOCl, CHX, OCT + EDTA and NaOCl + EDTA. The null hypothesis was rejected, because differences were found after using these root canal irrigants.

It was used a closed apical system to simulate a clinical condition, which is tooth's foramen and outer surface sealed by the periodontal ligament, and enclosed in alveolar bone (Parente et al., 2010). During irrigation protocol, an endodontic needle was inserted inside root canal 2 mm short of the working length. This needle insertion allows the root canal irrigant to reach the apical third and avoids the risk of extrusion of the root canal irrigant that would damage the periapical tissues (Teixeira, Felippe & Felippe, 2005) and because mechanical efficacy of irrigation at apical third is improved (Rödig et al., 2010). The root canals were instrumented up to ProTaper F4, with #40 apical diameter to allow adequate flow of irrigant to the working length (Schmidt et al., 2015; Urban, Donnermeyer, Schäfer & Bürklein, 2017).

There are currently questions about the evaluation of smear layer by SEM comparing the amount of open dentinal tubules in a single moment because the examiners are not able to determine the sclerotic dentin extension already present (Lottanti, Gautschi, Sener & Zehnder, 2009; Schmidt et al., 2015). Regarding this, it has been proposed in the literature the use of an experimental model in which root canal dentin is evaluated before and after the final irrigation. In this model, the teeth are instrumented, cleaved, and evaluated by SEM. Then, the teeth halves are placed back together, the specimens are irrigated with the final root canal irrigant and the post-treatment SEM images are obtained to evaluate the effects of the final irrigation (Schmidt et al., 2015). In the present study, this model was not used because it was evaluated the effect of root canal irrigants used during instrumentation and not as final irrigant. Additionally, a random distribution of specimens was used, for teeth of all groups to have the same distribution of sclerotic dentin. This avoids the bias when comparing the cleaning capacity of root canal irrigants.

OCT, NaOCl and CHX groups presented a poor cleaning capacity showing median of smear layer scores between 3 and 5, with no statistically significant difference. The poor cleaning capacity of CHX (Yamashita et al., 2003), as well as of NaOCl (Salman, Baumann, Hellmich, Roggendorf & Termaat, 2010) used as single irrigant has been related in the literature.

The smear layer is formed during biomechanical preparation and it is composed of inorganic and organic material and microorganisms (Violich & Chandler 2010). The removal of smear layer has been recommended (Lottanti et al., 2009, Schmidt et al., 2015) because it may prevent intracanal medications from penetrating into the root canal system and influence the adaptation (Violich & Chandler 2010), bond (Aranda-Garcia et al., 2013), and penetrability of root canal sealers into root dentin (Kokkas, Boutsioukis, Vassiliadis & Stavrianos, 2004). EDTA is the most commonly used chelating agent to remove the smear layer generated during root canal preparation (Violich & Chandler 2010). The effectiveness of NaOCl in association with EDTA in root canal cleaning showed in this study has been reported in literature (Haapasalo et al., 2014). There was no significant difference of smear layer scores between NaOCl + EDTA and OCT + EDTA groups, which had an effective cleaning of root canal.

In OCT, NaOCl + EDTA and OCT + EDTA groups there was greater removal of the smear layer in the cervical third than in the apical third. These results are in accordance with previous studies (Teixeira et al., 2005; Salman et al., 2010) which showed less ability to clean the apical third. This effect could be explained because at cervical third the canal diameter is higher than apical third, which allows a better circulation of root canal irrigant (Teixeira et al., 2005) and consequently better cleanness.

In conclusion, OCT used as root canal irrigant during instrumentation had no dentin cleaning capacity and should be used with a final irrigation with EDTA to obtain smear layer removal. Further studies are necessary to determine the biocompatibility and the effects on radicular dentin of OCT and OCT associated with EDTA.

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4 CONCLUSÃO

- Nas células do ligamento periodontal de humanos e nos fibroblastos L929, o OCT a 0,1% apresentou menor citotoxicidade do que a CHX a 2%, $Ca(OCl)_2$ a 2,5% e a 5% e NaOCl a 2,5%. $Ca(OCl)_2$ a 2,5% e a 5% apresentaram citotoxicidade menor ou similar ao NaOCl a 2,5% respectivamente. Portanto, do ponto de vista de citotoxicidade, o OCT e o $Ca(OCl)_2$ apresentam potencial para serem usados como irrigantes endodônticos (Publicação 1).

- OCT usado como irrigante durante a instrumentação do canal radicular não apresentou capacidade de limpeza da dentina e, portanto, deveria ser usado com irrigação final de EDTA para se obter remoção da *smear layer* (Publicação 2).

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[*] De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf

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APÊNDICE A – Material e método estendido.

Publicação 1

“Cytotoxicity of calcium hypochlorite and octenidine hydrochloride in L929 and human periodontal ligament cells”.

Preparo das soluções irrigadoras

Foram estudadas as soluções de $Ca(OCl)_2$ a 2,5% e 5% (Sigma-Aldrich, St. Louis, MO, EUA), OCT a 0,1% (Octenisept®, Schulke & Mayr, Nordersdedt, Alemanha), NaOCl a 2,5% (DinoCloro, AraQuímica, Araraquara, SP, Brasil) e CHX a 2% (Reativa Farmácia de Manipulação, Araraquara, SP, Brasil). Imediatamente antes do uso, a solução de $Ca(OCl)_2$ foi preparada a partir da dissolução do pó de $Ca(OCl)_2$ em água destilada, e a solução de NaOCl a 2,5% foi preparada a partir de uma solução de NaOCl na concentração de 9% determinada por meio de titulação. O teor de cloro disponível nas soluções de NaOCl e de $Ca(OCl)_2$ foi determinado pelo método físico-químico de espectofotômetria (Rice et al., 2012). As soluções de $Ca(OCl)_2$ nas concentrações de 2,5% e 5%, OCT a 0,1%, NaOCl a 2,5% e CHX a 2% foram consideradas grau de diluição 1 e foram submetidas a diluições seriadas em solução salina (0,9% de cloreto de sódio) utilizando o fator de diluição 1,5 (Viola et al., 2017). As células foram incubadas com soluções nas diluições de 1/111,06; 1/166,6; 1/250; 1/375; 1/562; 1/844; 1/1266 e 1/1898, que correspondem às doses/concentrações de 0,9%, 0,6%, 0,4%, 0,26%, 0,18%, 0,12%, 0,08% e 0,05%, respectivamente.

Cultura de células e protocolo de tratamento com as soluções

Foram utilizadas a linhagem celular permanente de fibroblastos de camundongo L929 (American Type Culture Collection) e células do hPDL. Todos os procedimentos foram realizados seguindo as diretrizes e regulamentos éticos aplicáveis do Comitê de Ética em Pesquisa da Faculdade, que aprovou o projeto (número 57134916.3.0000.5416; Anexo B). Terceiros molares humanos, sem evidência de lesões de cárie ou doença periodontal, foram obtidos de pacientes saudáveis de 16 a 25 anos que foram atendidos na clínica de cirurgia da Faculdade, após previa assinatura do termo de consentimento livre e esclarecido, no caso de pacientes maiores de idade, e, termo de assentimento livre e esclarecido assinado pelo responsável legal, no caso de pacientes menores de idade. Após a extração, os dentes foram imediatamente armazenados no meio Eagle Modificado por Dulbecco - DMEM (Sigma-Aldrich, St. Louis, MO, EUA). O ligamento periodontal foi removido do terço médio da superfície radicular utilizando uma lâmina de bisturi N° 15, fragmentado e cultivado em placas de cultura de 100 mm por meio da técnica de explante (Lin et al., 2004). Foram utilizadas células do hPDL da 3ª a 6ª passagem, e os ensaios foram realizados em triplicata usando as células de 3 doadores. Ambas células do hPDL e L929 foram cultivadas com DMEM suplementado com 10% de soro fetal bovino - SFB (Gibco/Invitrogen, Waltham, MS, EUA), 1% de penicilina e estreptomicina (Sigma-Aldrich) (100.00U/mL de penicilina, 100,00 mg/mL de estreptomicina), em estufa a 37ºC, com atmosfera umidificada contendo 5% de CO_2 e 95% de ar.

Para a realização dos ensaios, as células foram destacadas das garrafas com tripsina, centrifugadas a 1000 RPM por 10 minutos a 5ºC, o pellet foi resuspendido em DMEM com SFB 10% e as células foram contadas em hemocitômetro (Reichert, Bufallo, NY, EUA). As células foram cultivadas em placas de cultura de 24 ou 96 poços (Corning Inc., Corning, NY, EUA) contendo meio de cultura com SFB 10% e foram mantidas na estufa por 24 horas para aderirem nos poços. Em seguida, o meio de cultura foi removido e as células foram incubadas com diferentes doses das soluções estudadas por um período de 10 minutos (Viola et al., 2017). As soluções foram, então, removidas, e as células foram incubadas com o meio de cultura com SFB 10% durante 4 horas (Giannelli et al., 2008; Viola et al., 2017). A solução salina e o meio de cultura foram utilizados como controles.

Avaliação da viabilidade/metabolismo celular por ensaio de metil-tiazol-tetrazólio (MTT)

As células do hPDL ($8x10^4$ células/mL) e L929 ($6,5x10^4$ células/mL) foram cultivadas em placas de cultura de 96 poços (Corning). Após realizar o protocolo de tratamento com as soluções nas doses de 0,05-0,9% e com os controles, o meio de cultura foi removido e o ensaio MTT foi realizado. Foram adicionados 100µl da solução de MTT (Sigma-Aldrich) a 0,5mg/mL, sendo as células incubadas por 3 horas em estufa à 37°C contendo 5% de CO_2, 95% de umidade. Após este período, foram adicionados 100µL de álcool isopropílico acidificado (HCl: álcool isopropílico, 0.04N) ao extrato para solubilizar os cristais de formazan. As densidades ópticas foram medidas por espectrofotômetro com o filtro de 570nm de comprimento de onda (ThermoPlate, Nashan Distict, Shenzhen, China). As leituras de absorbância foram normalizadas com as leituras das células expostas à solução salina e representaram atividade de succinato desidrogenase (metabolismo celular). Os ensaios foram realizados em triplicata e repetidos em três períodos de tempo independentes.

Avaliação da viabilidade celular por ensaio do vermelho neutro (NR)

As células do hPDL ($8x10^4$ células/mL) e L929 ($6,5x10^4$ células/mL) foram cultivadas em placas de cultura de 96 poços (Corning). Após realizar o protocolo de tratamento com as soluções nas doses de 0,05-0,9% e com os controles, o meio de cultura foi removido, e foram adicionados 100µL da solução de NR (Sigma-Aldrich) a 0,05mg/mL, sendo as células incubadas por 3 horas em estufa com 5% de CO2, 95% de umidade a 37ºC. Após este período, a solução foi descartada e foram adicionados 100µL de solução de ácido acético a 1% em etanol a 50% em cada poço. As densidades ópticas das soluções foram medidas por espectrofotômetro com filtros de 570nm de comprimento de onda (ThermoPlate). As leituras de absorbância foram normalizadas com as leituras das células expostas à solução salina e representaram a capacidade de incorporar o corante pelos os lisossomos das células viáveis. Os ensaios foram realizados em triplicata e repetidos em três tempos independentes.

Teste de cicatrização (Scratch assay)

As células do hPDL ($5x10^5$ células / mL) e L929 ($2,5x10^5$ células / mL) foram cultivadas em placas de cultura de 24 poços (Corning) e foram mantidas em estufa a 37ºC, com 95% de umidade e 5% de CO_2 até confluência por 24 horas. Em seguida, células foram removidas com uma ponteira P200 (TPP, Techno Plastic Products, Trasadigen, Suíça) criando um sulco artificial no centro de cada poço, denominado “cicatriz” ou “ferida”. Imediatamente, as células foram lavadas 2 vezes com PBS e foram submetidas ao protocolo de tratamento com as soluções estudadas na dose 0,05% e com os controles.

Para determinar a área de migração celular, os poços foram fotografados às 0, 8, 16 e 24 horas no microscópio EVOS F1 (AMC, Bothell, WA, EUA) e as imagens foram analisadas de forma cega por dois examinadores calibrados usando o 145 ImageJ software (National Institutes of Health, NIH, Bethesda, Maryland, EUA). Os experimentos foram realizados em quadruplicata e repetidos em três tempos independentes. Foram fotografados e analisados 8 campos diferentes por poço (Liang et al., 2007).

Análise estatística

Os dados foram analisados por meio do programa estatístico Graph Pad Prism 5 (GraphPad Software, La Jolla, CA, EUA), empregando ANOVA de dois fatores e teste de Bonferroni com nível de significância de 5%.

Publicação 2

“Cleaning capacity of octenidine as root canal irrigant: a scanning electron microscopy study”

Preparação de espécimes

Todos os procedimentos foram realizados de acordo com as diretrizes éticas e os regulamentos do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Araraquara da Universidade Estadual Paulista (número 65131917.1.0000.5416; Anexo C).

Cinquenta dentes unirradiculados humanos foram radiografados digitalmente nas direções bucolingual e mesiodistal para conferir a presença de um único canal radicular e morfologia similar entre os dentes selecionados. Os critérios de exclusão foram: anatomia complexa do canal radicular, presença de calcificações, canais radiculares que permitiram a introdução de um instrumento superior ao tamanho #20 no forame apical, curvaturas apicais, reabsorção radicular externa ou interna ou tratamento endodôntico prévio. Os dentes selecionados foram armazenados em timol 0,1% até à sua utilização. As coroas dos dentes foram removidas e as raízes foram padronizadas no comprimento de 16 mm usando um disco diamantado operado em baixa velocidade. Para simular uma situação clínica, foi colocada resina composta (Z-100, 3M / ESPE, Salt Lake City, Utah) no ápice radicular para evitar a extrusão da solução irrigadora a partir do forame apical. O comprimento de trabalho do canal radicular foi estabelecido a 1 mm do forame apical. As raízes foram distribuídas aleatoriamente em 5 grupos de acordo com o protocolo de irrigação empregado: G1, OCT a 0,1% (Octenisept, Schülke & Mayr); G2, CHX a 2% (Arte & Ciência Farmácia de Manipulação, Araraquara, SP, Brasil); G3, NaOCl a 2,5% (Asfer Indústria Química Ltda., São Caetano do Sul, SP, Brasil); G4, OCT a 0.1% durante a instrumentação seguida de irrigação final com EDTA a 17% (Biodinâmica, Ibiporã, PR, Brasil) e G5, NaOCl a 2,5% durante a instrumentação, seguido de irrigação final com EDTA a 17%.

Os canais radiculares foram instrumentados com o sistema rotatório ProTaper (Dentsply Maillefer, Ballaigues, Suíça) até a lima F4 a uma velocidade de 300 rpm e torque de 1,5 - 3 Ncm, usando motor endodôntico elétrico (X-Smart Plus, Dentsply Maillefer, Ballaigues, Suíça). Entre cada lima, a irrigação foi realizada com 2 mL das soluções irrigantes no canal radicular durante 1 minuto. As soluções irrigadoras foram colocadas em seringas de 5 mL (Ultradent Products, South Jordan, UT) com uma agulha endodôntica 30G de saída lateral (Ultradent), que foi colocada no interior do canal radicular 2 mm aquém do comprimento de trabalho. Os dentes dos grupos que incluíram EDTA a 17% foram irrigados com 3 mL desta solução por 3 minutos. No final da instrumentação, todos os grupos foram irrigados com 3 mL de água destilada por 2 minutos para evitar o efeito residual das soluções irrigantes na dentina.

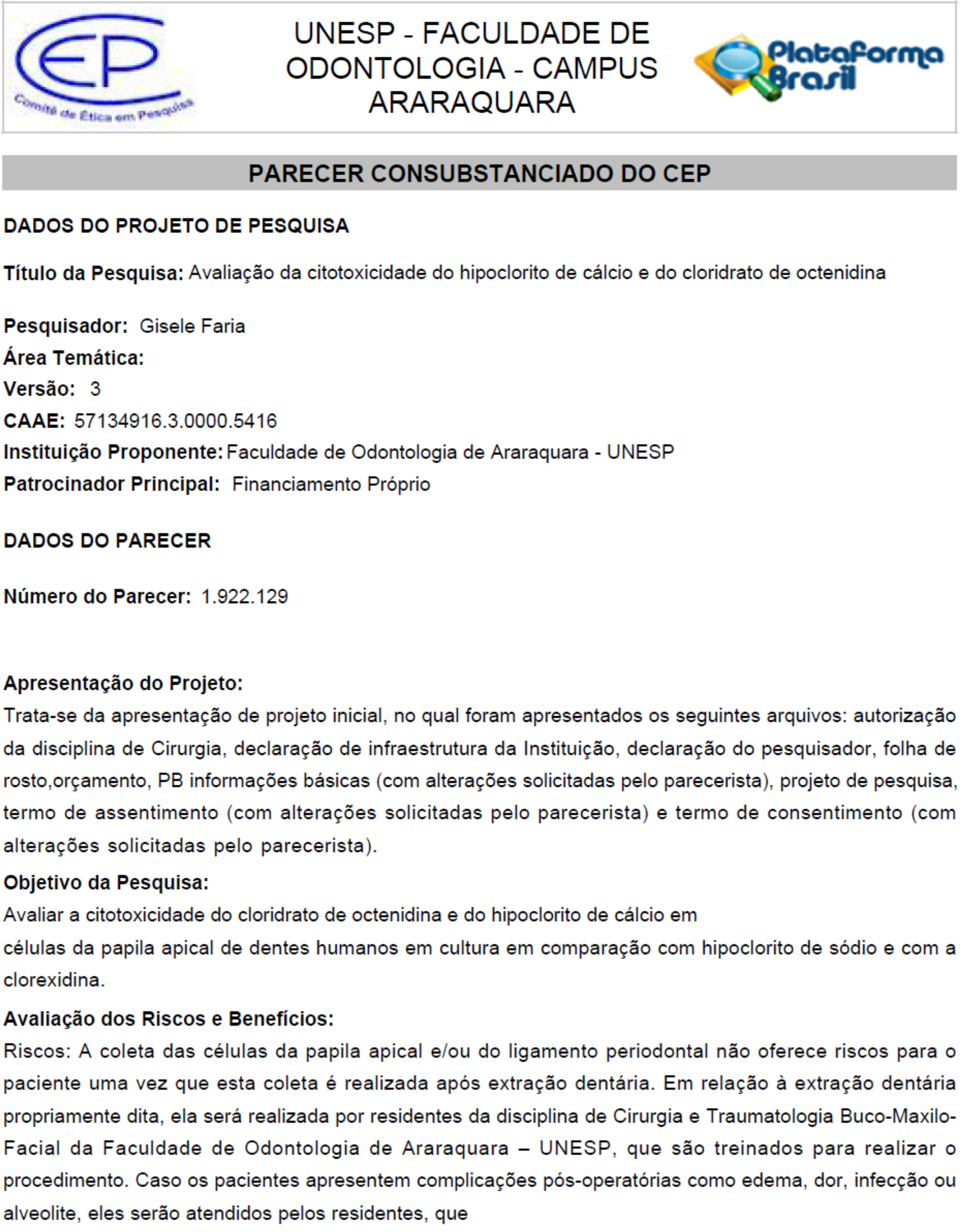
Avaliação em microscópio eletrônico de varredura (MEV)

Foram confeccionados sulcos longitudinais nas superfícies vestibular e lingual dos espécimes com disco de carburundum, preservando a camada interna da dentina ao redor do canal. As raízes foram, então, seccionadas usando um cinzel e um martelo. Todas as raízes foram seccionadas ao longo do seu eixo longitudinal, desidratadas, revestidas com uma camada de ouro de 20 nm e examinadas em microscópio electrónico de varredura (EVO 50, Zeiss, Cambridge, Reino Unido) a 20 Kv de acordo com a metodologia de Faria et al. (2014). Foram feitas fotomicrografias em aumentos de 2000X nos terços cervical, médio e apical do canal radicular. Para avaliar a capacidade de remoção de *smear layer* das soluções irrigantes foi utilizado o sistema de escores proposto por Hülsmann et al. (1997). As pontuações para o *smear layer* foram: 1, sem camada de *smear layer* com túbulos dentinários abertos; 2, pequena quantidade de *smear layer* com algum túbulo dentinário aberto; 3, camada de *smear layer* homogênea cobrindo a parede do canal radicular com apenas alguns túbulos dentinários abertos; 4, parede do canal radicular coberta completamente por uma camada homogênea de *smear layer* sem túbulos dentinários abertos; 5, camada pesada não homogênea de *smear layer* cobrindo completamente a parede do canal radicular. Todos os espécimes foram analisados independentemente por dois examinadores calibrados (Kappa > 0,9) que não sabiam a identificação dos grupos. Os escores foram então comparados, e quando houve alguma diferença, os examinadores inspecionaram conjuntamente o espécime até chegar um acordo sobre a pontuação.

Análise estatística

Os dados foram analisados empregando os testes de Kruskal-Wallis e Dunn com nível de significância de 5%, por meio do programa Graph Pad Prism 5 (Graph Pad Software In., San Diego, CA, EUA).

ANEXO A - Parecer consubstanciado do Comitê de Ética em Pesquisa referente ao projeto de pesquisa da Publicação 1 (Avaliação da citotoxicidade do hipoclorito de cálcio e do cloridrato de octenidina em diferentes linhagens celulares).



UNESP - FACULDADE DE
ODONTOLOGIA - CAMPUS
ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da citotoxicidade do hipoclorito de cálcio e do cloridrato de octenidina

Pesquisador: Gisele Faria
Área Temática:
Versão: 3
CAAE: 57134916.3.0000.5416
Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.922.129

Apresentação do Projeto:

Trata-se da apresentação de projeto inicial, no qual foram apresentados os seguintes arquivos: autorização da disciplina de Cirurgia, declaração de infraestrutura da Instituição, declaração do pesquisador, folha de rosto,orçamento, PB informações básicas (com alterações solicitadas pelo parecerista), projeto de pesquisa, termo de assentimento (com alterações solicitadas pelo parecerista) e termo de consentimento (com alterações solicitadas pelo parecerista).

Objetivo da Pesquisa:

Avaliar a citotoxicidade do cloridrato de octenidina e do hipoclorito de cálcio em
células da papila apical de dentes humanos em cultura em comparação com hipoclorito de sódio e com a clorexidina.

Avaliação dos Riscos e Benefícios:

Riscos: A coleta das células da papila apical e/ou do ligamento periodontal não oferece riscos para o paciente uma vez que esta coleta é realizada após extração dentária. Em relação à extração dentária propriamente dita, ela será realizada por residentes da disciplina de Cirurgia e Traumatologia Buco-Maxilo-Facial da Faculdade de Odontologia de Araraquara – UNESP, que são treinados para realizar o procedimento. Caso os pacientes apresentem complicações pós-operatórias como edema, dor, infecção ou alveolite, eles serão atendidos pelos residentes, que tomarão as providências para solucionar os problemas.

Benefícios: O benefício principal é obter informações para o conhecimento da citotoxicidade do cloridrato de octenidina e do hipoclorito de cálcio, e também
fornecer subsídios para a sua indicação ou não como solução irrigadora na clínica endodôntica.

Comentários e Considerações sobre a Pesquisa:

As adequações do TCLE e TALE e do PB Informações foram realizadas.

Considerações sobre os Termos de apresentação obrigatória:

As solicitações anteriores foram catadas. Histórico de solicitações:

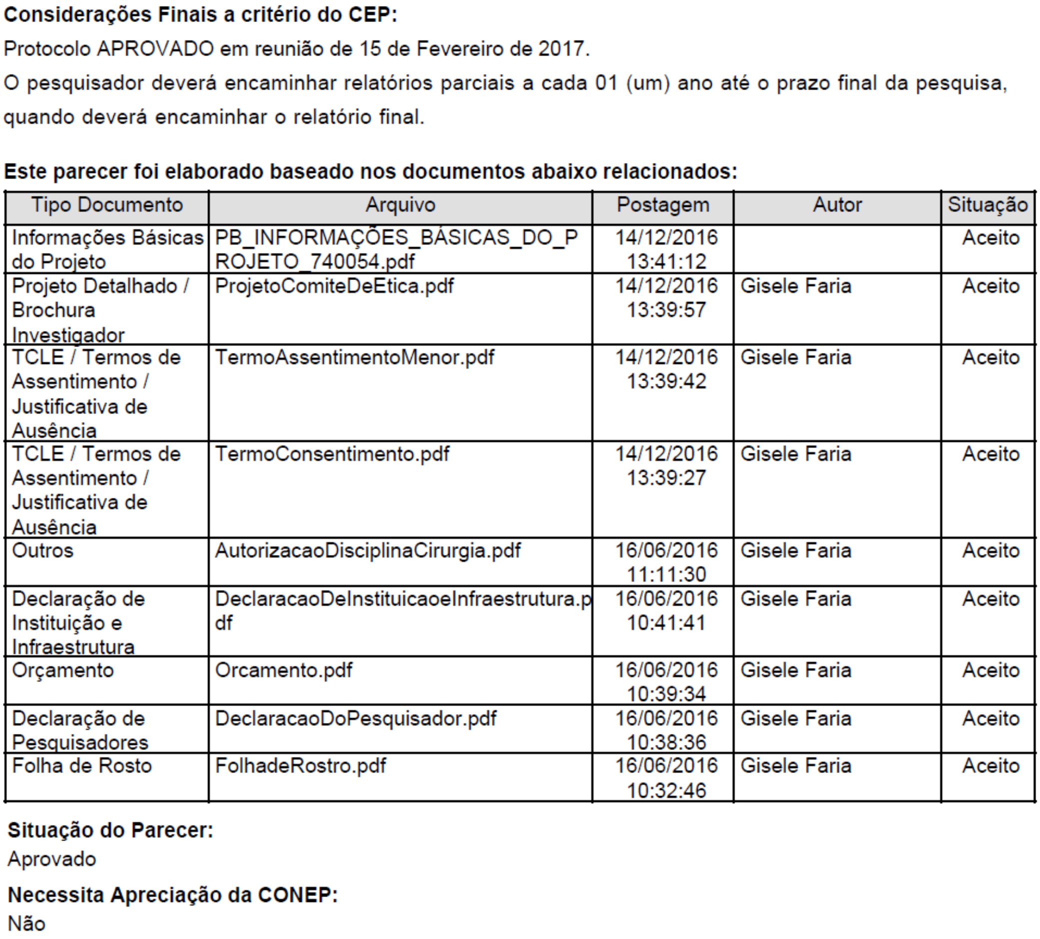
1º parecer: "No resumo faltou inserir o numero da amostra. O orçamento apresentado de R$11.020,00 é por conta do pesquisador responsável ou receberá apoio financeiro da Faculdade para realizar a pesquisa, pois está escrito que receberá apoio financeiro da Faculdade". O número da amostra foi inserido no resumo. O orçamento foi adequado para financiamento próprio. Os termos de apresentação obrigatória foram apresentados. O TCLE e
Termo de Assentimento deverão ser refeitos: 1 - precisa explicar para o leigo o seguinte: "objetivo desta pesquisa é coletar os tecidos da papila apical e/ou ligamento periodontal dos terceiros molares", 2 - A extração ocorrerá por motivos ortodonticos, então o seguinte texto deve ser repensado: "que serão extraídos no Centro Cirúrgico da Disciplina de Cirurgia e Traumatologia Buco-Maxilo-Facial da Faculdade de Odontologia de Araraquara – UNESP (FOAr), por indicação ortodôntica, para a realização deste trabalho de pesquisa,", pois tem a impressão que serão extraídos para a realização desta pesquisa, 3- Precisa esclarecer com detalhes a forma de acompanhamento: "Também fui informado quanto aos possíveis riscos durante a cirurgia e no pósoperatório, assim como da utilização de medicamentos e prestação de auxílios se necessário. As demais informações foram relatadas na anamnese., 4 - deixar claro no TCLE, os riscos e benefícios da pesquisa, quais procedimentos serão utilizados para a coleta dos tecidos da papila apical e/ou ligamento periodontal dos terceiros molares, forma de ressarcimento de despesas, destino do material biológico. Com relação aos itens solicitados no parecer anterior, os itens 1 e 2 foram contemplados. Os itens 3 e 4 não foram inseridos no TCLE e portanto precisam ser adequados, pois no PB Informações Básicas, e isso não consta do TCLE.

2º parecer: Solicitação de adequação dos itens 3 e 4 citados acima.

3º parecer e atual: os termos de apresentação foram adequados.

Conclusões ou Pendências e Lista de Inadequações:

Não existem pendências.



Considerações Finais a critério do CEP:

Protocolo APROVADO em reunião de 15 de Fevereiro de 2017.

O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da pesquisa, quando deverá encaminhar o relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_740054.pdf	14/12/2016 13:41:12		Aceito
Projeto Detalhado / Brochura Investigador	ProjetoComiteDeEtica.pdf	14/12/2016 13:39:57	Gisele Faria	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoAssentimentoMenor.pdf	14/12/2016 13:39:42	Gisele Faria	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoConsentimento.pdf	14/12/2016 13:39:27	Gisele Faria	Aceito
Outros	AutorizacaoDisciplinaCirurgia.pdf	16/06/2016 11:11:30	Gisele Faria	Aceito
Declaração de Instituição e Infraestrutura	DeclaracaoDeInstituicaoeInfraestrutura.pdf	16/06/2016 10:41:41	Gisele Faria	Aceito
Orçamento	Orcamento.pdf	16/06/2016 10:39:34	Gisele Faria	Aceito
Declaração de Pesquisadores	DeclaracaoDoPesquisador.pdf	16/06/2016 10:38:36	Gisele Faria	Aceito
Folha de Rosto	FolhadeRostro.pdf	16/06/2016 10:32:46	Gisele Faria	Aceito

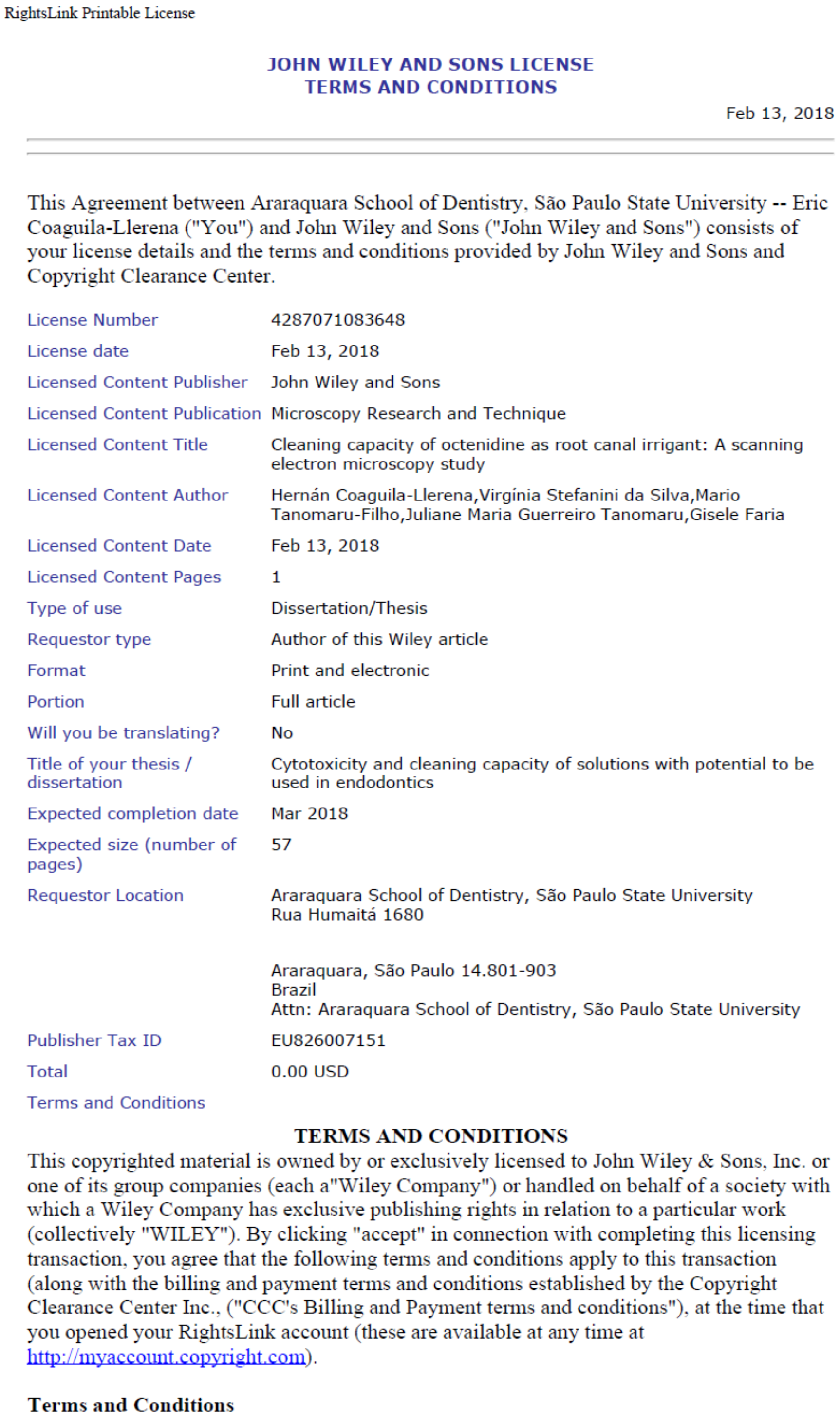
Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

ANEXO B - Autorização da editora *John Wiley & Sons Ltd.* para o uso do artigo "*Cleaning capacity of octenidine as root canal irrigant: a scanning electron microscopy study*", disponível no periódico "*Microscopy Research and Technique*".



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Licensed Content Title	Cleaning capacity of octenidine as root canal irrigant: A scanning electron microscopy study
Licensed Content Author	Hernán Coaguila-Llerena,Virgínia Stefanini da Silva,Mario Tanomaru-Filho,Juliane Maria Guerreiro Tanomaru,Gisele Faria
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Will you be translating?	No
Title of your thesis / dissertation	Cytotoxicity and cleaning capacity of solutions with potential to be used in endodontics
Expected completion date	Mar 2018
Expected size (number of pages)	57
Requestor Location	Araraquara School of Dentistry, São Paulo State University Rua Humaitá 1680 Araraquara, São Paulo 14.801-903 Brazil Attn: Araraquara School of Dentistry, São Paulo State University
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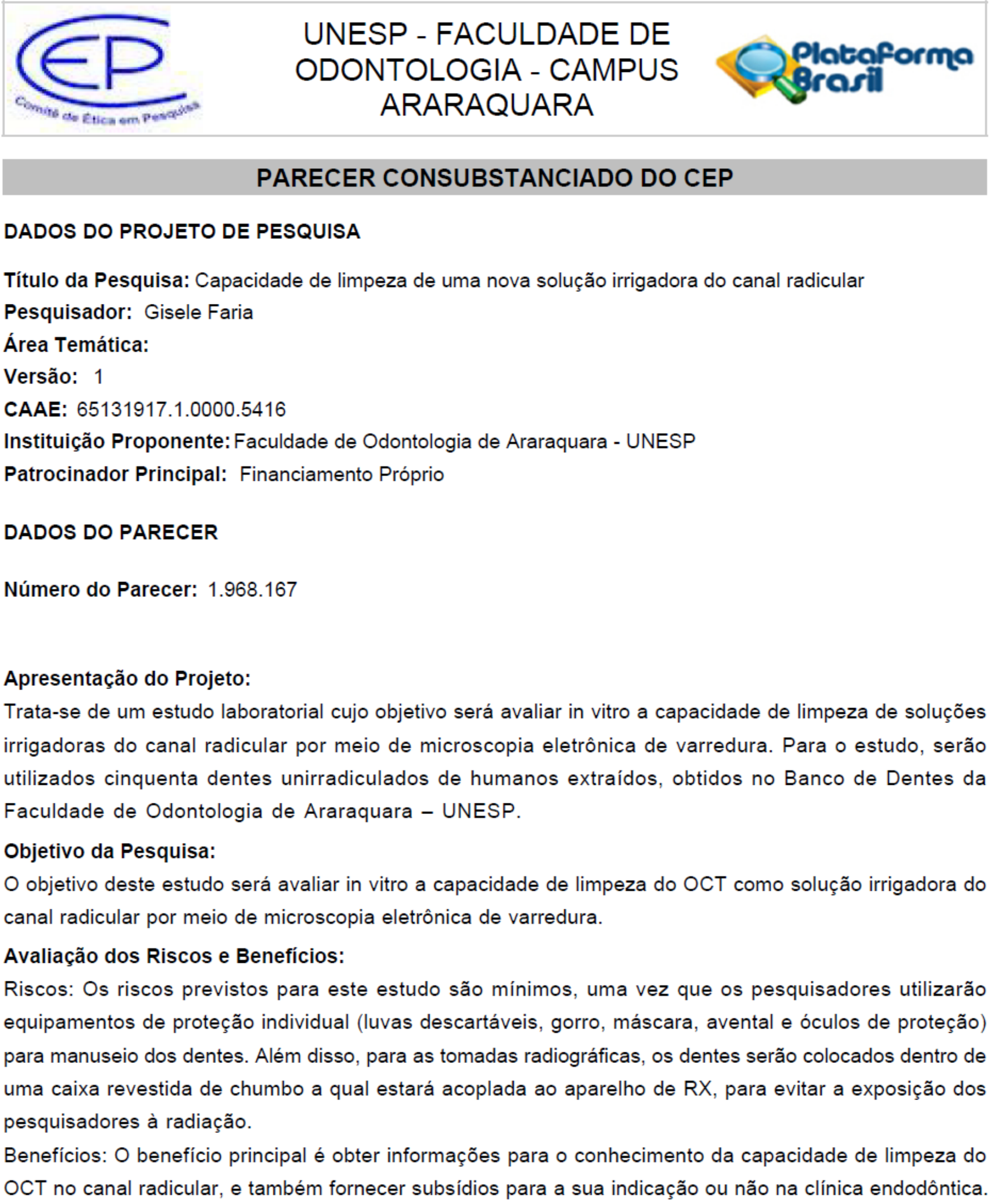
ANEXO C - Parecer consubstanciado do Comitê de Ética em Pesquisa referente ao projeto de pesquisa da Publicação 2 (Cleaning capacity of octenidine as root canal irrigant: A scanning electron microscopic study).



UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Capacidade de limpeza de uma nova solução irrigadora do canal radicular
Pesquisador: Gisele Faria
Área Temática:
Versão: 1
CAAE: 65131917.1.0000.5416
Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP
Patrocinador Principal: Financiamento Próprio

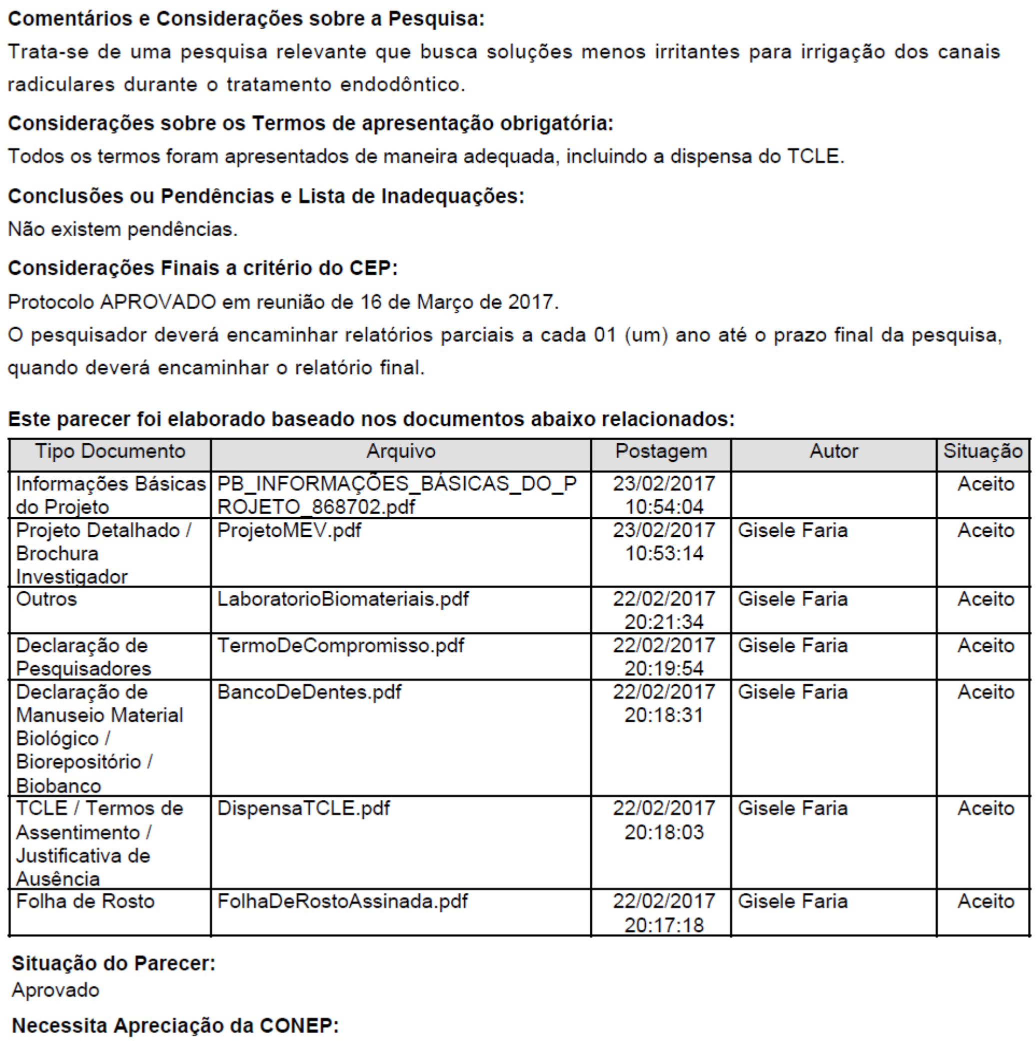
DADOS DO PARECER

Número do Parecer: 1.968.167

Apresentação do Projeto:
Trata-se de um estudo laboratorial cujo objetivo será avaliar in vitro a capacidade de limpeza de soluções irrigadoras do canal radicular por meio de microscopia eletrônica de varredura. Para o estudo, serão utilizados cinquenta dentes unirradiculados de humanos extraídos, obtidos no Banco de Dentes da Faculdade de Odontologia de Araraquara – UNESP.

Objetivo da Pesquisa:
O objetivo deste estudo será avaliar in vitro a capacidade de limpeza do OCT como solução irrigadora do canal radicular por meio de microscopia eletrônica de varredura.

Avaliação dos Riscos e Benefícios:
Riscos: Os riscos previstos para este estudo são mínimos, uma vez que os pesquisadores utilizarão equipamentos de proteção individual (luvas descartáveis, gorro, máscara, avental e óculos de proteção) para manuseio dos dentes. Além disso, para as tomadas radiográficas, os dentes serão colocados dentro de uma caixa revestida de chumbo a qual estará acoplada ao aparelho de RX, para evitar a exposição dos pesquisadores à radiação.
Benefícios: O benefício principal é obter informações para o conhecimento da capacidade de limpeza do OCT no canal radicular, e também fornecer subsídios para a sua indicação ou não na clínica endodôntica.



Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa relevante que busca soluções menos irritantes para irrigação dos canais radiculares durante o tratamento endodôntico.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram apresentados de maneira adequada, incluindo a dispensa do TCLE.

Conclusões ou Pendências e Lista de Inadequações:

Não existem pendências.

Considerações Finais a critério do CEP:

Protocolo APROVADO em reunião de 16 de Março de 2017.

O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da pesquisa, quando deverá encaminhar o relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_868702.pdf	23/02/2017 10:54:04		Aceito
Projeto Detalhado / Brochura Investigador	ProjetoMEV.pdf	23/02/2017 10:53:14	Gisele Faria	Aceito
Outros	LaboratorioBiomateriais.pdf	22/02/2017 20:21:34	Gisele Faria	Aceito
Declaração de Pesquisadores	TermoDeCompromisso.pdf	22/02/2017 20:19:54	Gisele Faria	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	BancoDeDentes.pdf	22/02/2017 20:18:31	Gisele Faria	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DispensaTCLE.pdf	22/02/2017 20:18:03	Gisele Faria	Aceito
Folha de Rosto	FolhaDeRostoAssinada.pdf	22/02/2017 20:17:18	Gisele Faria	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Araraquara, 21 de março de 2018.

Eric Hernán Coaguila Llerena