UNIVERSIDADE DE PASSO FUNDO

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Efeito do Hipoclorito de Cálcio e Ácido Glicólico sobre a Rugosidade, Relação Apatita/Colágeno e Resistência Flexural da Dentina

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Efeito do Hipoclorito de Cálcio e Ácido Glicólico na Rugosidade, Relação Apatita / Colágeno e Resistência à Flexão da Dentina

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da UPF, para obtenção do título de Doutora em Odontologia – Área de Concentração em Clínica Odontológica, sob orientação do Prof. Dr. **Doglas Cecchin**.

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% - Porcentagem

$Ca(OCl)_2$ - Hipoclorito de Cálcio

$Ca(OH)_2$ - Hidróxido de Cálcio

EDTA - Ácido Etilenodiamino Tetra-Acético

FTIR - Espectroscopia Infravermelha

GA - Ácido Glicólico

h - Hora

H2O2 - Peróxido de Hidrogênio

HOCl - Ácido Hipocloroso

min. - Minutos

ml - Mililitros

mm - Milímetros

NaOCl - Hipoclorito de sódio

pH - Potencial Hidrogeniônico

s - Segundos

RESUMO[1]

O objetivo deste estudo foi avaliar os efeitos do hipoclorito de cálcio ($Ca(OCl)_2$) e do ácido glicólico (AG) sobre a rugosidade, relação apatita/colágeno e resistência flexural da dentina mineralizada. No Artigo I as amostras foram divididas da seguinte forma: G1: solução fisiológica, G2: 2,5% NaOCl, G3: 2,5% $Ca(OCl)_2$, G4: 17% EDTA, G5: 10% AG, G6: 17% AG. Todas as soluções aumentaram significativamente a rugosidade dentinária do canal radicular quando comparadas ao grupo controle ($p > 0,001$). Entre os irrigantes NaOCl 2,5% e Ca $(OCl)_2$ 2,5%, ambos interferiram na relação apatita/colágeno, diferente do grupo controle ($P < 0,05$), mas não diminuíram a resistência flexural, sendo semelhante ao grupo controle ($P > 0,05$). No Artigo II as amostras foram divididas da seguinte forma: G1: solução fisiológica, G2: 2,5% NaOCl + 17% EDTA, G3: 2,5% NaOCl + 10% AG, G4: 2,5% NaOCl + 17% AG, G5: 2,5% $Ca(OCl)_2$ + 17% EDTA, G6: 2,5% $Ca(OCl)_2$ + 10% AG, G7: 2,5% $Ca(OCl)_2$ + 17% GA. Com exceção do protocolo NaOCl + 10% GA, todos os outros protocolos aumentaram significativamente a rugosidade dentinária do canal radicular quando comparados ao grupo controle ($p > 0,001$). O único protocolo que não interferiu na relação

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apatita/colágeno, semelhante ao grupo controle (P> 0,05) e não diminuir a resistência flexural, semelhante ao grupo controle (P> 0,05), foi o protocolo NaOCl + 17% GA. Este estudo apoia o uso do $Ca(OCl)_2$ 2,5% e do Ácido Glicólico, como soluções alternativas durante o preparo de canais radiculares e como protocolo alternativo para limpeza e desinfecção dos canais radiculares o estudo sugere NaOCl + 17% GA.

Palavras-chave: EDTA, Hipoclorito de sódio, dentina radicular, endodontia, dentina.

ABSTRACT[2]

The aim of this study was to evaluate the effects of calcium hypochlorite ($Ca(OCl)_2$) and glycolic acid (GA) on roughness, apatite/collagen ratio and flexural strength of mineralized dentin. In Article I, the main ones were divided as follows: G1: saline solution, G2: 2.5% NaOCl, G3: 2.5% $Ca(OCl)_2$, G4: 17% EDTA, G5: 10% AG, G6: 17% AG. All solutions increased the complete dentinal roughness of the root canal when compared to the control group ($p>0.001$). Among the irrigants NaOCl 2.5% and $Ca(OCl)_2$ 2.5%, both interfered in the apatite / collagen ratio, different from the control group ($P < 0.05$), but did not decrease the flexural strength, being similar to the group control ($P>0.05$). In Article II, the main items were divided as follows: G1: saline solution, G2: 2.5% NaOCl + 17% EDTA, G3: 2.5% NaOCl + 10% AG, G4: 2.5% NaOCl + 17% AG, G5: 2.5% $Ca(OCl)_2$ + 17% EDTA, G6: 2.5% $Ca(OCl)_2$ + 10% AG, G7: 2.5% $Ca(OCl)_2$ + 17% GA. With the exception of the NaOCl + 10% GA protocol, all other protocols increased to complement the dentinal roughness of the root canal when compared to the control group ($p>0.001$). The only protocol that did not interfere in the apatite/collagen ratio, similar to the

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control group (P>0.05) and did not decrease flexural strength, similar to the control group (P>0.05), was the NaOCl + 17% GA protocol. This study supports the use of 2.5% $Ca(OCl)_2$ and Glycolic Acid as alternative solutions during root canal preparation and as an alternative protocol for root canal cleaning and disinfection the right study NaOCl + 17% GA.

Palavras-chave: EDTA, Sodium hypochl, root dentin, endodontics, dentin.

INTRODUÇÃO

A limpeza e desinfecção com instrumentos endodônticos associados a irrigantes de canais radiculares é o principal objetivo do preparo dos canais radiculares (ZANDI *et al.*, 2016). O irrigante mais utilizado atualmente em endodontia é o NaOCl, por seu amplo espectro antimicrobiano (DU *et al.*, 2014; SIQUEIRA *et al.*, 1998) e sua capacidade de promover a dissolução do tecido orgânico (DU *et al.*, 2014; DUTTA *et al.*, 2012; SIQUEIRA *et al.*, 1998). Porém, apresenta algumas desvantagens, como citotoxicidade (TANOMARU FILHO *et al.*, 2002), interferência na adesão (SANTOS *et al.*, 2006) e instabilidade química (LEONARDO *et al.*, 2016).

O uso de limas endodônticas no canal radicular provoca deposição de *smear layer* nas paredes do canal, que mantém as bactérias e seus subprodutos na dentina radicular, impede a penetração de desinfetantes intracanais e cimentos nos túbulos dentinários e diminui a capacidade de selamento da dentina (SHAHRAVAN *et al.*, 2007). O ácido etilenodiaminotetracético (EDTA) é uma das soluções de irrigação mais utilizadas para a remoção da *smear layer* (CALT *et al.*, 2002; MANCINI *et al.*, 2009). O EDTA apresenta características desfavoráveis como desnaturação das fibrilas de colágeno (GARBEROGLIO *et al.*,

1994) e erosão dentinária peritubular e intertubular (ZEHNDER, 2006). Além disso, o EDTA é citotóxico e participa da toxicidade aquática (NOWACK *et al.*, 1996). Assim, novas soluções irrigantes têm sido estudadas para auxiliar no preparo quimio e mecânico de canais radiculares (BELLO *et al.*, 2018).

O hipoclorito de cálcio $Ca(OCl)_2$ mostrou ser eficaz contra *E. faecalis* quando utilizado como solução irrigante principal (BELLO *et al.*, 2018; DE ALMEIDA *et al.*, 2014), também promove a dissolução do tecido (DUTTA *et al.*, 2012), é quimicamente estável em armazenamento (LEONARDO *et al.*, 2016) e é mais biocompatível que o NaOCl (BLATTES *et al.*, 2017). Segundo DE ALMEIDA *et al.* (2014) $Ca(OCl)_2$ pode auxiliar no preparo quimio e mecânico, contribuindo de forma significativa para a redução do conteúdo microbiano durante o tratamento endodôntico. Na odontologia, estudos recentes mostraram que o Ácido Glicólico (AG) é adequado para o condicionamento do esmalte e dentina em procedimentos restauradores (CECCHIN *et al.*, 2019; CECCHIN *et al.*, 2018) e tão eficiente quanto o EDTA na remoção da *smear layer* das paredes dos canais radiculares (BARCELLOS *et al.*, 2020; BELLO *et al.*, 2020). BELLO *et al.* (2020) concluíram que o AG apresentou estabilidade de pH em todos os tempos e temperaturas avaliados; a relação apatita / colágeno diminuiu com o aumento das concentrações de AG; enquanto a resistência à flexão não foi significativamente afetada pela concentração de AG.

Portanto, o objetivo do presente estudo foi avaliar o efeito de diferentes protocolos de irrigantes endodônticos e as sustâncias isoladamente na rugosidade, relação apatita / colágeno e resistência à flexão da dentina mineralizada. Os irrigantes testados foram: 2,5% NaOCl e 2,5% $Ca(OCl)_2$ como solução irrigante principal; e, das soluções irrigantes finais: 17% EDTA, 10% AG e 17% AG. Este estudo testou a hipótese de que não haveria influência na rugosidade, relação apatita / colágeno e resistência à flexão após exposição ao $Ca(OCl)_2$ e AG.

PROPOSIÇÃO

Objetivos gerais

Avaliar o efeito de diferentes irrigantes sobre a dentina e seus componentes.

Objetivos específicos

- Avaliar a rugosidade dentinária, após exposição aos seguintes protocolos de irrigação: NaOCl/EDTA, NaOCl/AG 10%, NaOCl/AG 17%, $Ca(OCl)_2$/EDTA, $Ca(OCl)_2$/AG 10%, $Ca(OCl)_2$/AG 17%. Além disso, cada solução foi analisada isoladamente.

- Caracterizar a degradação química dos principais constituintes da dentina, por meio de FTIR, após exposição aos mesmos protocolos de irrigação citados acima.

- Examinar a resistência flexural da dentina após exposição aos mesmos protocolos de irrigação citados acima.

Hipóteses em estudo:

Não haveria alteração na rugosidade dentinária após exposição ao $Ca(OCl)_2$ e o AG.

Não haveria degradação química dos principais constituintes da dentina após exposição ao $Ca(OCl)_2$ e o AG.

Não haveria influência na resistência à flexão da dentina após exposição ao $Ca(OCl)_2$ e ao AG.

ARTIGO I

Effect of Calcium Hypochlorite and Glycolic Acid on Roughness, Apatite/Collagen Ratio and Flexural Strength of Dentin[3]

Abstract: The aim of this study was to evaluate the effects of calcium hypochlorite ($Ca(OCl)_2$) and glycolic acid (GA) on the chemical and mechanical properties of dentin to investigate the potential use of these substances as root irrigants. Samples were divided into seven groups according to decontamination protocol: G1: Saline solution, G2: 2,5% NaOCl, G3: 2,5% $Ca(OCl)_2$, G4: 17% EDTA, G5: 10% GA, G6: 17% GA. Specifically, alterations in roughness, apatite/collagen ratio and flexural strength of mineralized dentin were evaluated. Saline was used as a negative control. The surface roughness (Ra, µm) of the canal lumen was measured on each specimen held in a horizontal position on a rugosimeter. The apatite/collagen (A/C) ratio in the dentin powder was

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examined by Fourier transform infrared spectroscopy (FTIR). Dentin beams were used for 3-point bending strength (σ) test. The results of Ra, A/C and σ were statistically analyzed using ANOVA and Tukey tests ($\alpha = 0.05$). All solutions significantly increased the dentinal roughness of the root canal when compared to control group ($p>0.001$). Among the irrigants NaOCl 2.5% and $Ca(OCl)_2$ 2.5%, both interfered with the apatite/collagen ratio, different to the control group ($P<0.05$), but did not decrease the flexural strength, similar to the control group ($P>0.05$). Among the final irrigants, 15% EDTA was the only substance capable of not interfering with the apatite/collagen ratio, similar to the control group ($P>0.05$) and not decreasing flexion resistance, similar to the control group ($P>0.05$). Therefore, this study supports the potential use of NaOCl 2.5% and $Ca(OCl)_2$ 2.5%, as an alternative irrigation solution, and 17% EDTA as an alternative final irrigation for root canal preparation.

Keywords: EDTA, Sodium hypochl, root dentin, endodontics, dentin.

1. Introduction

The main objective of the root canals preparation is cleaning and disinfection using endodontic instruments associated with root canal irrigants [1]. NaOCl is the most commonly used irrigant in endodontics because of its broad antimicrobial spectrum [2-4] and its ability to promote organic tissue dissolution [2, 5]. However, it does have some disadvantages, such as cytotoxicity [6], interference in adhesion [7] and chemical instability [8]. Ethylenediaminetetraacetic acid (EDTA) is one of the most commonly used irrigation solution for removal of the smear layer [9, 10]. EDTA it has unfavorable features such as denaturation of collagen fibrils [11] and peritubular and intertubular dentin erosion [12]. Furthermore, the EDTA is cytotoxic, and plays a part in aquatic toxicity [13]. Thus, newer irrigants solutions have been studied to aid in the chemomechanical preparation of root canals [14].

$Ca(OCl)_2$ has been found to be effective against *E. faecalis* [14, 15], also promotes tissue dissolution [5], chemically stable in storage [8] and is more biocompatible than NaOCl [16]. According to de Almeida, Souza, Miyagaki, Dal Bello, Cecchin and Farina [15] $Ca(OCl)_2$ can aid in chemomechanical preparation, contributing in a significant way to the reduction of microbial content during root canal treatament. For final irrigantion, the glycolic acid (GA) has showed ability for smear layer removal from root canal walls [17, 18]. Furthermore, Bello, Farina, Souza and Cecchin [18] concluded that GA showed pH stability at all times and temperatures evaluated; the apatite/collagen ratio reduced with increased GA concentrations; while flexural strength was not significantly affected by GA concentration.

Therefore, the objective of the present study was to evaluate the effect of different intraradicular irrigants on roughness, apatite/collagen ratio and flexural strength of mineralized dentin. The irrigants tested were: 2,5% NaOCl and 2,5% $Ca(OCl)_2$ as main irrigant solution; and, of the finals irrigants solutions: 17% EDTA, 10% GA, and 17% GA. This study tested the hypothesis that there would no influence on roughness, apatite/collagen ratio and flexural strength after exposure to $Ca(OCl)_2$ and AG.

2 Material and Methods

2.1. Experimental design

This study was approved by the ethical institutional review board of a local university (#4.596.792). Human extracted teeth third molars were selected for this study. The adherent soft tissues were cleaned and all the teeth were stored in saline solution at 4 °C until further tests.

For the treatment of dentin samples, the 2,5% $Ca(OCl)_2$, 10% GA and 17% GA (Natupharma, Passo Fundo, Rio Grande do Sul, Brazil) solutions were prepared. As a positive control, NaOCl (Natupharma, Passo Fundo, Rio Grande do Sul, Brazil) was selected as the main endodontic irrigant the NaOCl, and 17% EDTA as a final irrigant solution (Biodinamica, Ibipora, Parana, Brazil). Saline solution was used to treat samples in the negative control group.

2.2. Roughness

Nine human mandibular third molars were used for the roughness test. Mid-coronal dentin disks were cut perpendicular to the longitudinal axis of each tooth with a slow-speed diamond saw under constant water-cooling. Each tooth resulted in an average of two discs of dentin, the segments were divided into 06 groups (n=03). The dentin surfaces were flattened using silicon carbide paper (500, 800, 1000, and 1200 grit) under constant water irrigation, and polished using a suspension of 0.1-mm alumina on a rotating felt disc.

In Group 1 (control) without treatment, the samples were stored in saline solution. The Group 2 and 3, the samples were immersed in chemical solution for 30 minutes (renewed every 03 minutes); after irrigated with 5 mL of saline solution and kept moist until tested. From Group 4 to group 6, the samples were immersed in the final irrigator for 1 minute; washed with saline solution and kept moist until tested (table 1).

Table 1. Classification of groups according to irrigation protocol
Groups
G1 - Saline solution
G2 - 2,5% NaOCl (2 ml – 30 minutes - 20ml)
G3 – 2,5% $Ca(OCl)_2$ (2 ml – 30 minutes - 20ml)
G4 - 17% EDTA (2 ml – 1 minutes)
G5 - 10% GA (2 ml – 1 minutes)
G6 - 17% GA (2 ml – 1 minutes)

The surface roughness (Ra, μm) of the canal lumen was measured in each specimen maintained at horizontal position in a rugosimeter (Mitutoyo SJ-410, Kanagawa, Japan); the mean value of Ra was determined as the average of those at three randomly selected areas (1×1 mm²). The mean and standard deviation of Ra was determined for the entire surface roughness which is defined as the arithmetic mean of all absolute distances of the roughness profile from the centerline within the measured length [19]. The data were not normally distributed ($p < 0.05$) and hence, transformed into the square root and analyzed using ANOVA and Tukey test ($p < 0.05$).

2.3. Apatite/collagen ratio using Fourier-transform infrared spectroscopy

Six human mandibular third molars were selected for this test. Enamel and cementum were removed from the teeth using a diamond bur #2215 in a high-speed handpiece under refrigeration. Dentin powder (90 µm) was obtained with a high-speed handpiece and diamond bur #3145 F without refrigeration. The powder went through a 90 µm sieve, so the dentin grains were equal to or smaller than this size. The dentin powder was divided in six groups, of 9 mg each. For irrigation, dentin powder was placed over a filter paper and fixed on a glass Becker. The irrigations were performed with a 25×4 mm needle, following the protocol described in the table 1. After irrigation, the powder was air dried at 37 °C [17].

FTIR spectra of the dentin powder were collected for each group ($n = 3$). Spectra were obtained between 650 and 4000 cm^{-1} resolution, using 48 scans (Agilent Cary; 630 FTIR spectrometer, Santa Clara, USA). The IR spectrum is produced as a result of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of chemical bonds within a molecule. Thereby, when IR radiation is absorbed by an organic molecule, it is converted into molecular vibration energy, and the spectrum portray the vibrational motion and usually appears in the form of bands [20]. The range for the characterization of organic compounds is mid-IR (4000 to 400 cm^{-1}) [21].

Apatite/collagen ratios derived from FTIR spectroscopy showed normal distribution using the Shapiro-Wilk Normality test ($p = 0.882$) and Equal Variance test ($p = 0.419$). Data were statistically analyzed using one-way ANOVA and Tukey's post-hoc test for multiple comparison ($\alpha = 0.05$).

2.4. Flexural strength test

Thirty human mandibular third molars were used for the flexural strength test. Mid-coronal dentin disks were cut perpendicular to the longitudinal axis of each tooth with a slow-speed diamond saw under constant water-cooling. The disks were trimmed to a final rectangular-shaped beam (5.0 mm length, 0.2 mm thick, 2.0 mm wide) (20). Two beams were obtained from each tooth, totaling 60 beams, which were randomly divided into six groups ($n = 10$) following the irrigation protocols previously mentioned (table 1).

Flexural tests were conducted using a three-point flexure device with a 3-mm support span. The specimens were tested at a crosshead speed of 0.5 mm/min using a universal testing machine (Instron, Canton, Norwood, USA). Flexural strength (in MPa) was calculated using the following equation: 3PL/2bd2, where P = load at fracture (N), L = length of support span (mm), b = beam width (mm) [17].

Flexural strength data was normally distributed as observed in the Shapiro-Wilk Normality test ($p = 0.05$) and Equal Variance test ($p = 0.405$). Data were statistically analyzed using one-way ANOVA and Tukey's post-hoc test for multiple comparison ($\alpha = 0.05$).

3 Results and Discussion

3.1. Roughness

The mean roughness and standard deviation are summarized in Table 2. The highest dentin roughness was found in the 2.5% $Ca(OCl)_2$, 17% EDTA and 10% GA irrigants, which were statistically similar between them ($P>0.05$), followed by 2.5% NaOCl and 17% GA which were statistically similar between them ($P>0.05$) and finally the control group which differs statistically from the other groups and originated the lowest dentin roughness ($P<0.05$).

Dentin is a substrate with a complex organic and inorganic structure. This substrate is composed of 22% by weight of hydrated organic matrix, most of which consists of type I collagen and an inorganic reinforcement phase of carbonated apatite that contributes considerably to its mechanical properties [22]. NaOCl is known to be a non-proteolytic product specific agent that is capable to act on organic matter, as well as magnesium and carbonate ions [23]. Thus, NaOCl breaks long peptide chains and chlorin terminal protein groups, consequently, can affect the roughness of the dentin through the degradation of organic components. The result of the roughness of $Ca(OCl)_2$ can be explained by its low solubility causing a slow release of chlorine with a higher content at the end of its action [5], in addition to the release of two molecules of hypochlorous acid during its action [14] while NaOCl releases only one molecule [15].

All final irrigation solutions showed an increase in surface roughness compared to the control group ($P<0.05$). As previously mentioned, dentin demineralization is closely associated with roughness, and EDTA has the ability to combine with calcified dentin components through a chelating mechanism, resulting in demineralization and softening of the tissue [12, 24], coinciding with our results. In previous study, GA showed a decrease in dentinal microhardness comparable to phosphoric acid[25] and was less aggressive in promoting enamel demineralization than PA in previous study [25]. The rough surfaces allow a clinical benefit in the micromechanical bonding of the adhesive materials, which requires the presence of surface irregularities of the adherent into which the adhesive can penetrate [19].

Kataoka, Sasaki, Hidalgo, Nakano and Shimizu [26] reported that when GA is applied for the purpose of skin exfoliation, it has easy penetration compared to other alpha hydroxyl acids like CA and malic acid, due to the small size of its molecule. Therefore, softening effects on the dentinal walls with microhardness reduction and increasing roughness, can be advantageous in the clinic however, the degree of softening and demineralization may have an influence on the physical and chemical properties of this structure [19, 27].

Table 1. Roughness, Apatite/Collagen Ratios Derived from FTIR Spectroscopy and Flexural Strength Values Obtained with the 3-Point Flexure Test

Groups	Roughness	Apatite/collagen ratio	Flexural strength (MPa)
Saline	0.13 ± 0.01^{c}	1.20 ± 0.86^{b}	5.39 ± 0.60^{a}
2,5% NaOCl	0.15 ± 0.04^{b}	3.09 ± 1.48^{a}	5.81 ± 0.63^{a}
2,5% $Ca(OCl)_2$	0.24 ± 0.10^{a}	2.21 ± 0.61^{a}	5.45 ± 0.50^{a}
17% EDTA	0.22 ± 0.03^{a}	1.03 ± 0.17^{b}	5.7 ± 0.48^{a}
10% GA	0.22 ± 0.03^{a}	0.99 ± 1.23^{b}	3.38 ± 1.22^{b}

17% GA	0.15 ± 0.03^{b}	0.63 ± 0.25^{c}	3.27 ± 0.43^{b}
Means followed by different letters in the same column are statistically different ($P<0.001$).			

3.2. Fourier-transform infrared spectroscopy

Amide bands I, II, and III from the intact collagen component of mineralized dentin and phosphate and carbonate bands from the apatite component were identified in the infrared (IR) spectrum. For evaluation of test substances, we selected the apatite/collagen ratio between the amide I peak at 1636cm^{-1} and the phosphate (u_3PO_4) peak at 1011 cm^{-1} (Fig. 1). This method was previously described [28]. The spectra of the mineralized dentin shown in Fig. 1 provides evidence of characteristics that are similar to those presented in other studies [28, 29]; thus, the method for obtaining the dentin powder presented in this research can be reproduced.

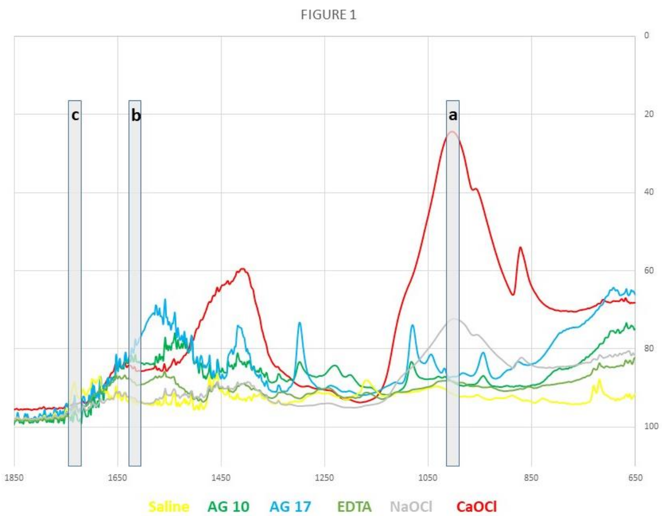

Figure 1. FTIR spectra of dentin powder after irrigation with the experimental solutions. Phosphate (a), amide I (b), and (c) peaks were used to obtain the apatite/collagen ratio.

The apatite/collagen ratio was affected by the use of NaOCl and $Ca(OCl)_2$ as observed in table 2. These findings are in agreement with Zhang, Kim, Cadenaro, Bryan, Sidow, Loushine, Ling, Pashley and Tay [28], since the use of NaOCl before EDTA and GA can promote collagen degradation and/or extraction and decrease the ratio apatite/collagen.

The apatite/collagen ratio was reduced with 17% GA, which showed considerable changes in the peaks of amides I, II and III, which may indicate that the action of this acid occurs in the organic and inorganic components of dentin. 17% EDTA and 10% GA showed similar results to the control group. Nevertheless, according to results presented previously [28], the results of IR spectra should be considered with caution because the depth penetration of IR radiation in the attenuated total reflection method is in the order of a few microns [28].

3.3 Flexural Strength

Flexural strength is important to maintain dentin properties, preventing the weakening of root dentin and reducing the risk of dentinal cracks [28, 30]. The mean flexural strength the solutions with de values and standard deviation are summarized in Table 2. 10% GA and 17% GA had a lowest flexural strength than the control group.

The 10% and 17% GA showed considerable changes in the flexion test, with statistical difference with the other solutions. GA has shown potential for microhardness reduction, dentin demineralization and smear layer removal [25]. In addition, it is important to link FTIR spectral results to mechanical evaluations to provide more consistent results. The degree of softening and demineralization can influence the physical and chemical properties of dentin, which can increase the risk of vertical tooth fracture [30]. Alterations in the apatite/collagen ratio presented by chelating agents, mainly by 17% AG, may indicate probable alterations in the surface properties of dentin. The only chelating substance that altered the apatite/collagen ratio and significantly decreased resistance was 17% AG.

4 Conclusions

Based on the findings of the present study, it can be concluded that all solutions used in this study increased the dentinal roughness of the root canal. However, among the irrigants NaOCl 2.5% and $Ca(OCl)_2$ 2.5%, both interfered with the apatite/collagen ratio, but did not decrease the flexural strength, presenting similar clinical applicability. Among the final irrigants, 17% EDTA was the only substance capable of not interfering with the apatite/collagen ratio, and did not reduce flexion resistance, being the best final irrigant with clinical applicability.

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Compliance with Ethical Standards

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Ethical Approval: All applicable international, national, and/or institutional guidelines for the care. This article does not contain any studies with human participants or animals performed by any of the authors. Extracted human teeth were used in this study. This study was approved by the University of Passo Fundo ethics committee (protocol No. 4.596.792).

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ARTIGO II

Evaluation of Different Root Canal Irrigant Protocols using Calcium Hypochlorite and Glycolic Acid on Roughness, Apatite/Collagen Ratio and Flexural Strength of Dentin [4]

Abstract: The aim of this study was to evaluate the effects of calcium hypochlorite ($Ca(OCl)_2$) and glycolic acid (GA) on the chemical and mechanical properties of dentin to investigate the potential use of these substances as root irrigants. Samples were divided into seven groups according to decontamination protocol: G1: Saline solution, G2: 2,5% NaOCl + 17% EDTA, G3: 2,5% NaOCl + 10% GA, G4: 2,5% NaOCl + 17% GA, G5: 2,5% $Ca(OCl)_2$ + 17% EDTA, G6: 2,5% $Ca(OCl)_2$ + 10% GA, G7: 2,5% $Ca(OCl)_2$ + 17% GA. Specifically, alterations in roughness, apatite/collagen ratio and flexural strength of mineralized dentin were evaluated. Saline was used as a negative control. The surface roughness (Ra, μm) of the canal lumen was measured on each specimen

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held in a horizontal position on a rugosimeter. The apatite/collagen (A/C) ratio in the dentin powder was examined by Fourier transform infrared spectroscopy (FTIR). Dentin beams were used for 3-point bending strength (σ) test. The results of Ra, A/C and σ were statistically analyzed using ANOVA and Tukey tests ($\alpha = 0.05$). With the exception of the NaOCl + 10% GA protocol, all other protocols were significantly increased the dentinal roughness of the root canal when compared to control group ($p>0.001$). However, the only protocol capable of not interfering with the apatite/collagen ratio, similar to the control group ($P>0.05$) and not decreasing flexion resistance, similar to the control group ($P>0.05$), was NaOCl + 17% GA protocol. It can be concluded that NaOCl + 17% GA protocol has ability to increase the dentinal roughness of the root canal, without interfering with the apatite/collagen relationship and decreasing flexural strength. Therefore, this study supports the potential use of NaOCl + 17% GA as protocol for root canal preparation.

Keywords: EDTA, Sodium hypochl, root dentin, endodontics, dentin.

1. Introduction

The cleaning and disinfection using endodontic instruments associated with root canal irrigants is the main objective of the root canals preparation [1]. The most commonly used irrigant in endodontics is the NaOCl, because of its broad antimicrobial spectrum [2-4] and its ability to promote organic tissue dissolution [2, 5]. However, it does have some disadvantages, such as cytotoxicity [6], interference in adhesive procedure [7] and it has chemical instability [8].

The using endodontic files in the root canal results causes deposition of a smear layer on the canal walls, that keeps bacteria and their byproducts in the root dentin, hinders the penetration of intracanal disinfectants and cements into the dentinal tubules, and decreases the sealing capacity of root canal sealers [9]. Ethylenediaminetetraacetic acid (EDTA) is one of the most commonly used irrigation solutions for removal of the smear layer [10, 11]. EDTA it has unfavorable features such as denaturation of collagen fibrils [12] and peritubular and intertubular dentin erosion [13]. Furthermore, the EDTA is cytotoxic, and plays a part in aquatic toxicity [14]. Thus, newer irrigants solutions have been studied to aid in the chemomechanical preparation of root canals [15].

Calcium Hypochlorite $Ca(OCl)_2$ has been found to be effective against *E. faecalis* when used as main irrigant solution [15, 16], also promotes tissue dissolution [5], chemically stable in storage [8] and is more biocompatible that NaOCl [17]. According to de Almeida, Souza, Miyagaki, Dal Bello, Cecchin and Farina [16] $Ca(OCl)_2$ can aid in chemomechanical preparation, contributing in a significant way to the reduction of microbial content during root canal treatament. In dentistry, recent studies showed GA to be suitable for enamel and dentin etching in restorative procedures [18-20] and as efficient as EDTA in removing smear layer from root canals walls [21, 22]. Bello, Farina, Souza and Cecchin [23] concluded that GA showed pH stability at all times and temperatures evaluated; the apatite/collagen ratio reduced with increased GA concentrations; while flexural strength was not significantly affected by GA concentration.

Therefore, the objective of the present study was to evaluate the effect of different endodontic irrigants protocols on roughness, apatite/collagen ratio and flexural strength of mineralized dentin. The irrigants tested were: NaOCl and $Ca(OCl)_2$ as main irrigant solution; and, of the finals irrigants solutions: 17% EDTA, 10% GA, and 17% GA. This study tested the hypothesis that there is no influence on roughness, dentin degradation, and flexural strength after exposure to $Ca(OCl)_2$ and AG.

2 Material and Methods

2.1. Experimental design

This study was approved by the institutional review board of a local university (#4.596.792). Human extracted teeth third molars were selected for this study. The adherent soft tissues were cleaned and all the teeth were stored in saline solution at 4 °C until further tests.

For the treatment of dentin samples, the $Ca(OCl)_2$ 2,5%, GA 10% and GA 17% (Natupharma, Passo Fundo, Rio Grande do Sul, Brazil) solutions were prepared. As a positive control, NaOCl 2,5% (Natupharma, Passo Fundo, Rio Grande do Sul, Brazil) was selected as the main endodontic irrigant, and 17% EDTA (Biodinamica, Ibipora, Parana, Brazil) was selected as final endodontic irrigant solution. Saline solution was used to treat samples in the negative control group.

2.2. Roughness

Eleven human mandibular third molars were used for the roughness test. Mid-coronal dentin disks were cut perpendicular to the longitudinal axis of each tooth with a slow-speed diamond saw under constant water-cooling. Each tooth resulted in an average of two discs of dentin, the segments were divided into 07 groups (n=03). The dentin surfaces were polished using silicon carbide paper (500, 800, 1000, and 1200 grit) under constant water irrigation. Final polishment was made using a suspension of 0.1-mm alumina on a rotating felt disc.

In Group 1 (control) without treatment, the samples were stored in saline solution. From Group 2 to group 7, the samples were immersed in main chemical solution for 30 minutes (renewed every 03 minutes); after irrigated with 5 mL of saline solution, an immersed again was made in the final irrigator for 1 minute; washed with saline solution and kept moist until tested (table 1).

The surface roughness (Ra, μm) of the canal lumen was measured in each specimen maintained at horizontal position in a rugosimeter (Mitutoyo SJ-410, Kanagawa, Japan); the mean value of Ra was determined as the average of those at three randomly selected areas (1×1 mm^2). The mean and standard deviation of Ra was determined for the entire surface roughness which is defined as the arithmetic mean of all absolute distances of the roughness profile from the centerline within the measured length [24]. The data were not normally distributed (p <

0.05) and hence, transformed into the square root and analyzed using ANOVA and Tukey test ($p < 0.05$).

Table 1. Classification of groups according to irrigation protocol
Groups
G1 - Saline solution
G2 - 2,5% NaOCl (2 ml – 30 min – 20 ml) + 17% EDTA (2 ml – 1 min)
G3 - 2,5% NaOCl (2 ml – 30 min – 20 ml) + 10% GA (2 ml – 1 min)
G4 - 2,5% NaOCl (2 ml – 30 min – 20 ml) + 17% GA (2 ml – 1 min)
G5 – 2,5% $Ca(OCl)_2$ (2 ml – 30 min – 20 ml) + 17% EDTA (2 ml – 1 min)
G6 – 2,5% $Ca(OCl)_2$ (2 ml- – 30 min – 20 ml) + 10% GA (2 ml – 1 min)
G7 – 2,5% $Ca(OCl)_2$ (2 ml -– 30 min – 20 ml) + 17% GA (2 ml – 1 min)

2.3. Apatite/collagen ratio using Fourier-transform infrared spectroscopy

Six human mandibular third molars were selected for this test. Enamel and cementum were removed from the teeth using a diamond bur #2215 in a high-speed handpiece under refrigeration. Dentin powder (90 µm) was obtained with a high-speed handpiece and diamond bur #3145 F without refrigeration. The powder went through a 90 µm sieve, so the dentin grains were equal to or smaller than this size. The dentin powder was divided in twelve groups, of 9 mg each. For irrigation, dentin powder was placed over a filter paper and fixed on a glass Becker. The irrigations were performed with a 25×4 mm needle, following the protocol described in the previous test and table 1. After irrigation, the powder was air dried at 37 °C [21].

FTIR spectra of the dentin powder were collected for each group ($n = 3$). Spectra were obtained between 650 and 4000 cm^{-1} resolution, using 48 scans (Agilent Cary; 630 FTIR spectrometer, Santa Clara, USA). The IR spectrum is produced as a result of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of chemical bonds within a molecule. Thereby, when IR radiation is absorbed by an organic molecule, it is converted into molecular vibration energy, and the spectrum portray the vibrational motion and usually appears in the form of bands [25]. The range for the characterization of organic compounds is mid-IR (4000 to 400 cm^{-1}) [26].

Apatite/collagen ratios derived from FTIR spectroscopy showed normal distribution using the Shapiro-Wilk Normality test ($p = 0.882$) and Equal Variance test ($p = 0.419$). Data were statistically analyzed using one-way ANOVA and Tukey's post-hoc test for multiple comparison ($\alpha = 0.05$).

2.4. Flexural strength test

Thirty-five human mandibular third molars were used for the flexural strength test. Mid-coronal dentin disks were cut perpendicular to the longitudinal axis of each tooth with a slow-speed diamond saw under constant water-cooling. The disks were trimmed to a final rectangular-shaped beam (5.0 mm length, 0.2 mm thick, 2.0 mm wide) [21]. Two beams were obtained from each tooth, totaling 70 beams, which were randomly divided into four groups ($n = 10$) following the irrigation protocols previously mentioned (table 1).

Flexural tests were conducted using a three-point flexure device with a 3-mm support span. The specimens were tested at a crosshead speed of 0.5 mm/min using a universal testing machine (Instron, Canton, Norwood, USA). Flexural strength (in MPa) was calculated using the following equation: $3PL/2bd^2$, where P = load at fracture (N), L = length of support span (mm), b = beam width (mm) [21].

Flexural strength data was normally distributed as observed in the Shapiro-Wilk Normality test ($p = 0.05$) and Equal Variance test ($p = 0.405$). Data were statistically analyzed using one-way ANOVA and Tukey's post-hoc test for multiple comparison ($\alpha = 0.05$).

3 Results and Discussion

3.1. Roughness

The average roughness and the solutions associated with the values and standard deviation are summarized in Table 2. The higher dentin roughness was found at $Ca(OCl)_2$ + EDTA protocol ($P<0.05$), followed by $Ca(OCl)_2$ + 10% GA and $Ca(OCl)_2$ + 17% GA protocols that was similar statistically between them ($P>0.05$); NaOCl + 17% GA ($P<0.05$) and NaOCl + 17% EDTA ($P<0.05$), protocols that was similar statistically between them and NaOCl + 10% GA that was similar to the control group ($P>0.05$).

EDTA enhances the removal of the smear layer in the root canal, increasing the chemical interaction of the irrigating solutions with the dentin walls [27]. GA associated with the irrigating solution reduces dentinal roughness and microhardness, but does not change the mineral composition of the dentin surface [21]. Agreeing with the results of this study, since the higher dentin roughness was found at $Ca(OCl)_2$ + EDTA protocol ($P<0.05$), followed by $Ca(OCl)_2$ + 10% GA and $Ca(OCl)_2$ + 17%. To further complement these results, Dutta and Saunders [5] observed that with $Ca(OCl)_2$ there is initially a slow decrease in available chlorine due to its low solubility, and after dissociation there is a greater availability of chlorine content than with the NaOCl solution.

When analyzing the groups with NaOCl, the greatest dentin roughness with this irrigating solution was found in the protocol NaOCl + 17% GA, followed by NaOCl + 17% EDTA; NaOCl + 10% GA, which was similar to the control group. NaOCl reacts mainly with the organic matrix of the dentin, increasing its permeability to the chelating agent EDTA, which in turn acts on the inorganic matrix, resulting in an increase in surface roughness [28]. However, in this study, both 17% solutions, EDTA and AG significantly increased dentin roughness, possibly due to their demineralizing power.

Table 2. Associated solutions: Roughness, Apatite/Collagen Ratios Derived from FTIR Spectroscopy and Flexural Strength Values Obtained with the 3-Point Flexure Test

Groups	Roughness	Apatite/collagen ratio	Flexural strength (MPa)
Saline	0.13 ± 0.01^{e}	1.20 ± 0.86^{b}	5.39 ± 0.60^{a}
NaOCl + 17% EDTA	0.18 ± 0.02^{d}	1.28 ± 0.39^{b}	4.60 ± 0.40^{b}
NaOCl + 10% GA	0.13 ± 0.02^{e}	0.51 ± 0.13^{c}	5.38 ± 0.63^{a}
NaOCl + 17% GA	0.27 ± 0.05^{c}	1.30 ± 0.54^{b}	5.98 ± 0.52^{a}
$Ca(OCl)_2$ + 17% EDTA	0.44 ± 0.09^{a}	1.37 ± 0.43^{b}	3.77 ± 0.91^{c}
$Ca(OCl)_2$ + 10% GA	0.33 ± 0.02^{b}	0.56 ± 0.29^{c}	4.65 ± 0.64^{b}
$Ca(OCl)_2$ + 17% GA	0.35 ± 0.02^{b}	2.87 ± 0.88^{a}	4.72 ± 0.42^{b}

Means followed by different letters in the same column are statistically different ($P<0.001$).

3.2. Fourier-transform infrared spectroscopy

Amide bands I, II, and III from the intact collagen component of mineralized dentin and phosphate and carbonate bands from the apatite component were identified in the infrared (IR) spectrum. For evaluation of test substances, we selected the apatite/collagen ratio between the amide I peak at 1636cm^{-1} and the phosphate (u_3PO_4) peak at 1011 cm^{-1} (Fig. 1). This method was previously described [29]. The spectra of the mineralized dentin shown in Fig. 1 provides evidence of characteristics that are similar to those presented in other studies [29, 30]; thus, the method for obtaining the dentin powder presented in this research can be reproduced.

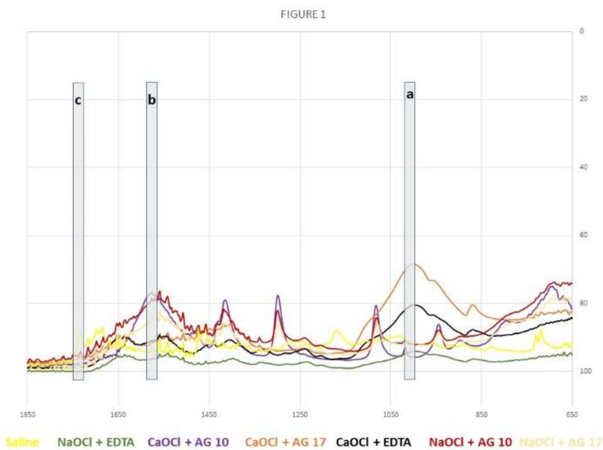

Figure 1. FTIR spectra of dentin powder after irrigation with the experimental solutions. Phosphate (a), amide I (b), and (c) peaks were used to obtain the apatite/collagen ratio.

The lowest values of the apatite / collagen ratio were presented in the protocols NaOCl + 10% GA and $Ca(OCl)_2$ + 10% GA, which showed similar results (Table 2). The main changes generated for NaOCl + 10% GA and $Ca(OCl)_2$ + 10% GA were verified in the phosphate peak (a) (Fig. 3). As aminomethyl propanol was used to obtain a solution of 10% GA, this substance may have reacted with the mineral and formed salts 20, reflecting the significant changes in the apatite/collagen ratio found in this study. However, it is important to mention that the infrared spectrum is formed as a result of the absorption of electromagnetic radiation and the conversion into molecular energy in the form of bands. Nevertheless, the depth of penetration of IR radiation in the ATR execution is in the order of a few microns [31] and the FTIR results should be considered with caution. $Ca(OCl)_2$ + 17% GA increased the proportion of collagen apatite. Therefore, according to the FTIR Ca (OCl) 2 + 17% GA is a protocol that, as it does not change the phosphate peak, does not form salts and does not react with the dentin mineral.

3.3 Flexural Strength

The flexural strength and the solutions associated with the values and standard deviation are summarized in Table 2. The lowest flexural strength was found in the protocol $Ca(OCl)_2$ + EDTA ($P < 0.05$), followed by NaOCl + 17% EDTA, $Ca(OCl)_2$ + 10% GA and $Ca(OCl)_2$ + 17% GA protocols that were statistically similar between them ($P > 0.05$), NaOCl + 10% GA ($P < 0.05$) and NaOCl + 17% GA similar to the control group ($P > 0.05$).

Flexural strength is important for maintaining dentin properties, avoiding root dentin embrittlement, and reducing the risk of dentinal cracks [29, 32]. By analyzing the groups that significantly decreased flexural strength, we can remember that EDTA as a final irrigant associated with an irrigant solution has the ability to combine with calcified dentin components through a chelating mechanism, resulting in demineralization and softening of the tissue [13, 33] confirming the results of the $Ca(OCl)_2$ + EDTA ($P < 0.05$) and NaOCl + 17% EDTA groups.

4 Conclusions

Based on the findings of the present study, it can be concluded that, with the exception of the NaOCl + 10% GA protocol, all other protocols increased the dentinal roughness of the root canal. However, the only protocol capable of not interfering with the apatite/collagen ratio, not decreasing flexion resistance and being a possible protocol with clinical applicability is NaOCl + 17% GA.

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Compliance with Ethical Standards

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Informed consent: Informed consent was not obtained because there were no human participants included in the study.

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CONSIDERAÇÕES FINAIS

Ao analisarmos as ações das soluções testadas neste estudo, procuramos levar em consideração o aumento da rugosidade, pois permitem um benefício clínico tanto na desinfecção dos canais e na ação micromecânica dos materiais adesivos, preservando a relação apatita/colágeno e resistência à flexão da dentina evitando complicações estruturais futuras.

Portanto ao analisarmos os efeitos das substâncias testadas, Ca(OCl)2 e o AG sobre a rugosidade, relação apatita/colágeno e resistência flexural da dentina mineralizada, podemos concluir que tanto a substância $Ca(OCl)_2$ e o protocolo NaOCl + 17% GA, são alternativas durante o preparo de canais radiculares, considerando.

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APÊNDICES

Parecer consubstanciado do CEP

UNIVERSIDADE DE PASSO FUNDO/ VICE-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO - VRPPG/ UPF

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DA AÇÃO DE DIFERENTES IRRIGANTES INTRARRADICULARES SOBRE OS COMPONENTES DENTINÁRIOS

Pesquisador: DEBORA PEREIRA DINIZ CORREIA BARCELLOS

Área Temática:

Versão: 2

CAAE: 42959620.2.0000.5342

Instituição Proponente: UNIVERSIDADE DE PASSO FUNDO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.596.792

Apresentação do Projeto:

Durante o preparo de canais radiculares é indicado o uso do Hipoclorito de Sódio (NaOCl) e EDTA com o objetivo de remover a smear layer. Além de serem citotóxicos aos tecidos orais e ao meio ambiente, podem fragilizar a estrutura dental levando à fratura radicular.

Objetivo da Pesquisa:

O estudo tem como objetivo avaliar a ação do Hipoclorito de Cálcio e do Ácido Glicólico (AG) sobre a dentina e seu componentes, traçando um comparativo com o NaOCl e o EDTA.

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores, a pesquisa não apresenta riscos uma vez que serão utilizados dentes humanos extraídos e obtidos através de doação. Como benefício citam que o Ácido Glicólico possui um atraente potencial capaz de agir em diferentes componentes da smear layer da parede de canais radiculares e trazer diversos benefícios para o tratamento endodôntico.

Comentários e Considerações sobre a Pesquisa:

O estudo é uma pesquisa experimental laboratorial com dentes humanos. Para avaliação do efeito das substâncias sobre os componentes dentinários será utilizado: Fourier transform infrared spectrometer (FTIR), resistência flexural da dentina e Microscopia de Transmissão (TEM). Para o primeiro teste, será obtido o pó de dentina de doze terceiros molares humanos submetidos ao tratamento com diferentes substâncias. Outros 120 terceiros molares humanos, serão fatiados e

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barras retangulares de dentina serão confeccionadas, as quais serão divididas em 12 grupos (n=10), de acordo com a substância experimental. As barras serão submetidas ao teste de flexão.

Considerações sobre os Termos de apresentação obrigatória:

O protocolo foi instruído e apresentado de maneira completa e adequada. Os compromissos do pesquisador e das instituições estavam presentes. O projeto foi considerado claro em seus aspectos científicos e metodológicos.

Recomendações:

Após o término da pesquisa, o CEP UPF solicita: a) A devolução dos resultados do estudo aos sujeitos da pesquisa ou a instituição que forneceu os dados; b) Enviar o relatório final da pesquisa, pela plataforma, utilizando a opção, no final da página "Enviar Notificação"+ relatório final.

Conclusões ou Pendências e Lista de Inadequações:

Diante do exposto, este Comitê, de acordo com as atribuições definidas na Resolução n. 466/12, do Conselho Nacional da Saúde, Ministério da Saúde, Brasil, manifesta-se pela aprovação do projeto de pesquisa na forma como foi proposto.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1677587.pdf	12/03/2021 13:44:00		Aceito
Outros	declaracaobiobanco.pdf	12/03/2021 13:43:35	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
Outros	autorizacaopelolocal.pdf	12/03/2021 13:42:09	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
Folha de Rosto	folhaderostoassinada.pdf	18/12/2020 22:05:38	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
Declaração de Pesquisadores	termodecompromissoparautilizacaodedados.pdf	07/12/2020 18:57:32	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
Solicitação Assinada pelo Pesquisador Responsável	declaracaobioseguranca.pdf	07/12/2020 18:49:35	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito

Declaração de Pesquisadores	declaracaonaoiniciodepesquisa.pdf	07/12/2020 18:48:07	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	declaracaodoacaodentes.pdf	07/12/2020 18:47:25	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	termodeconsentimentolivreesclarecido.pdf	07/12/2020 18:47:11	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito
Projeto Detalhado / Brochura Investigador	projetodeboradiniz.pdf	07/12/2020 18:45:56	DEBORA PEREIRA DINIZ CORREIA BARCELLOS	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PASSO FUNDO, 17 de Março de 2021

Assinado por:
Felipe Cittolin Abal
(Coordenador(a))