Antibacterial capacity of different intracanal medications on *Enterococcus faecalis*

Juliana Ferreira **PIOVESANI**[1]
Alex **SEMENOFF-SEGUNDO**[2]
Fábio Luiz Miranda **PEDRO**[2]
Álvaro Henrique **BORGES**[2]
Alessandra Nogueira Porto **NEVES**[2]
Iussif **MAMEDE NETO**[3]
Tereza Aparecida Delle Vedove **SEMENOFF**[2]

ABSTRACT

Objective: To evaluate the antimicrobial activity of 2% chlorhexidine gel, copaiba oil, propolis extract and calcium hydroxide associated to propylene glycol on *Enterococcus faecalis*. **Methods:** Fifty single-rooted human teeth were prepared until K-file #50 and distributed into groups according to the intracanal substances. The positive control group was only propylene glycol. Subsequently, 100µL of microorganism broth was inoculated into the roots, except in the negative control group. Then the roots were placed in individual test tubes immersed in BHI and put into incubator at 37° C for 48 hours. After the turbidity of the medium, the roots were irrigated with sodium thiosulfate, filled with one of the test substances and immersed in BHI for 7 days at 37° C. Thereafter, the drugs were removed from the roots with help of K-files and abundant irrigation with sodium thiosulfate. The roots were immersed in BHI for 24 hours. After this, the tubes were analyzed by single trained examiner to the categorization of the culture medium turbidity. **Conclusion:** According to this methodology, it was possible to conclude that none substance was effective against *Enterocossus faecalis*.

Keywords: Calcium hydroxide. Chlorhexidine. Enterococcus faecalis. Intracanal dressing. Propolis.

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[1]Specialist in Endodontics, Cuiabá University.
[2]MSc Professor in the Integrated Dentistry Science course, Cuiabá University.
[3]Specialist in Endodontics, UniABO/GO. MSc in Biology, ICB/UFG. Head of Specialization course in Endodontics, UniABO/MA. Coordinator of Endodontics discipline, UNIP/DF.

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Contact address: Tereza Aparecida Delle Vedove Semenoff
Rua Prof. Azélia Mamoré de Melo, 318 – Apto 63
Zip code: 78.005-700 – Araés – Cuiabá/MT – Brazil
E-mail: t.semenoff@uol.com.br

Introduction

The success of the endodontic treatment is closely related to the disinfection of the root canal system, obtained by the emptying, enlargement, action of the irrigant solution and intracanal dressing.[1,2,3] The chemico-mechanical preparation is effective to reduce the microbiota in the root canal lumen, however the microorganisms located in some areas of the canal, such as the non-mechanically prepared walls by the endodontic instruments, dentin tubules, isthmus, lateral canals and apical ramifications can show treatment-resistant due to the biofilm organization.[5,6] Persistent apical lesions that not reduced after the appropriate endodontic treatment are caused by the permanence of the pathogenic bacteria in the inner of the root canal system.[1]

Approximately 700 specimens of microorganisms compose the oral microflora.[7,8] *Enterococcus faecalis* are facultatively anaerobic bacteria, gram positive; forming a part of the intestinal microflora and commonly present in infections, for example, urinary tract and endocardium.[9,10] Rarely is found in cases of primary endodontic infections, but in cases of retreatment, represents between 38 to 70% of the microbiota.[9,10] Plays important role in the etiology of the endodontic infection in function of the particular strategy in the biofilm formation, virulence factors, adhesion to dentin collagen, survival to critic medium and resistance to endodontic therapy.[11]

Endodontic irrigants and intracanal dressing with antimicrobial action develop an important role in the way of reducing or completely eliminating the niches of bacterial colonization.[12] In this context, the calcium hydroxide, used in Dentistry since the beginning of twentieth century, presents an excellent ability to contribute in the repair of periapical lesions, anti-exsudative action and activity of induction the mineralization.[13] Chlorhexidine gluconate solution, in function of its substantivity and large antimicrobial spectrum has been proposed for this purpose, however due to its concentration of 2% (toxic to the most living tissues), inability of the inactivation of bacterial LPS and pulpal tissue dissolution, needs more rationale to be duly indicated.[2,14] However, the complete eradication of the microbiota in the root canal is not guaranteed with the use of these substances, needing the search for new medications for this purpose.[15]

Recently, products with natural origin are gaining space in the dentistry,[16,17] most of this related to undesirable properties of the classic solutions used with this objective.[18] One of these substances, the extract of propolis is the major substance in healthcare because of its therapeutic properties.[15,16,17] The antimicrobial characteristic is related to the presence of flavonoids and esters in its composition, in addition to being approximately 10 times less cytotoxic than the calcium hydroxide.[19] The copaiba oil, used a long time ago in empirical way by the indigenous people, spurred the interest of the scientific community, resulting in studies that confirmed its antibacterial activity.[20,21] Considering the importance of searching new medicaments for the endodontic therapy, the present study aims to evaluate the antibacterial effect of the extract of propolis and copaiba oil against to *Enterococcus faecalis*.

Material and methods

Fifty single-rooted human teeth, extracted by orthodontic reasons, were selected and prepared by manual technique until to K-file #50 (Maillefer Dentsply, Switzerland). The working length was determined to one millimeter before the radicular vertice. The root canal were irrigated with sodium hypochlorite 1% (Biodinâmica, Ibiporã, Brazil). After the biomechanical preparation, the crowns were removed and the root autoclaved to 121° C for 15 minutes. The specimens were randomly distributed in groups, with 10 units each,

Figure 1. Test substances: 1) Calcium hydroxide, 2) Extract of propolis, 3) Copaiba oil, 4) Chlorhexidine gluconate gel 2%, 5) Propylene glycol.

in accordance to the test substance (Fig 1), described in Table 1. Ten teeth, distributed equally, formed the positive control group, with propylene glycol, and the negative control group, without microorganism and intracanal dressing. The study was approved by the Ethics and Research Comitee of the University of Cuiabá with protocol number 2011-066.

In sequence, the roots, in the test groups, were inoculated with the *Enterococcus faecalis* (ATCC 29212) broth, with the pipette (Kacil Indústria e Comércio Ltda, Recife, Brazil) of fixed volume patterned with 100μL (Fig 2). All of roots were put in the 8 cm test tubes containing 5 mL of BHI culture medium (Newprov, Pinhais, Brazil) (Fig 3A) and taken to incubator, at 37° C for 48 hours, for the contamination of the dentin tubules. In the end of the period, the tubes were removed for the verification of the medium turbidity, parameter to confirm the microorganism growth in the culture medium (Fig 3B). In the next step, the samples were removed from the culture medium and washed with 10 mL of sodium thiosulfate 10% (Quimesp Química Ltda, Guarulhos, Brazil) (Fig 3C). The external drying was proceeded with a gauze and sterilized absorbent paper cones #50 (Dentsply, Petrópolis, Brazil) (Fig 3D) used for inner purpose.

The calcium hydroxide paste (Biodinâmica, Ibiporã, Brazil) was prepared in a toothpaste consistence and inserted in the roots of the CH group, by a K-file #25 (Maillefer Dentsply, Switzerland) (Fig 4A).

Table 1. Test substances and manufacturers.

Substance	Manufacturers
Copaiba Oil (CO)	Flores e Ervas Produtos Naturais Ltda.
Extract of Propolis (EP)	Flores e Ervas Produtos Naturais Ltda.
Calcium Hydroxide (CH)	Biodinâmica Química e Farmacêutica Ltda.
Chlrohexidine gluconate 2% (C)	Biológica Comércio e Manipulação de Medicamentos Ltda.
Propylene glycol (P)	Biológica Comércio e Manipulação de Medicamentos Ltda.

Figure 2. Inoculation of root canals in test groups with the broth of *Enterococcus faecalis* (ATCC 29212) through the pipette.

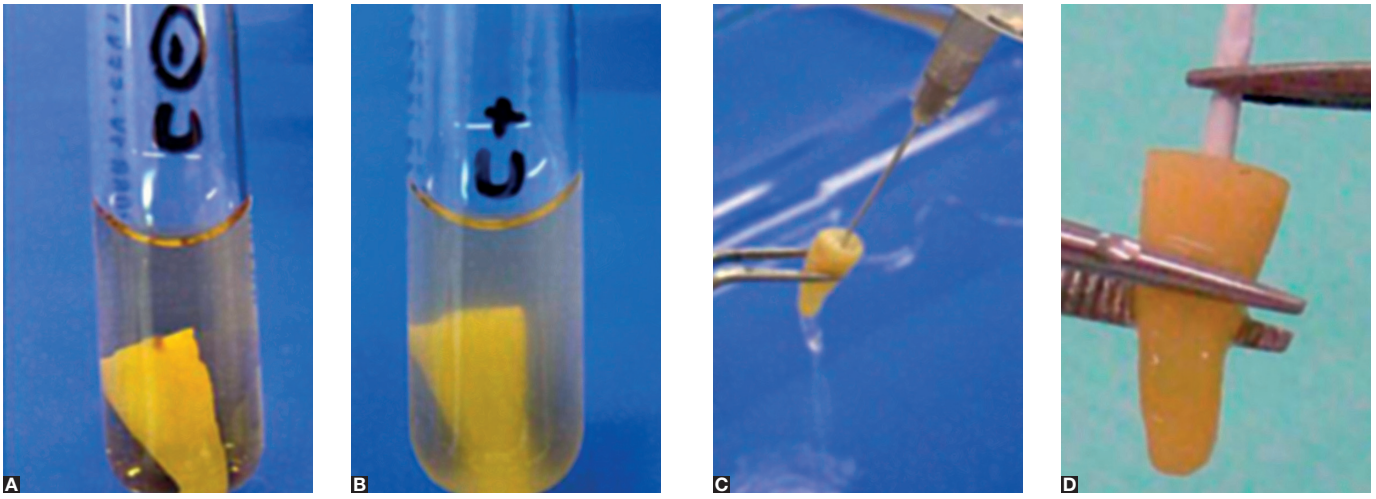

Figure 3. A) Roots in test tubes, containing 5 mL of BHI culture medium. **B**) Verification of medium turbidity, parameter to confirm microorganism growth of the culture medium. **C**) Samples washed with 10 mL of sodium thiosulfate 10% **D**) Drying of the roots with absorbent sterilized paper cones # 50.

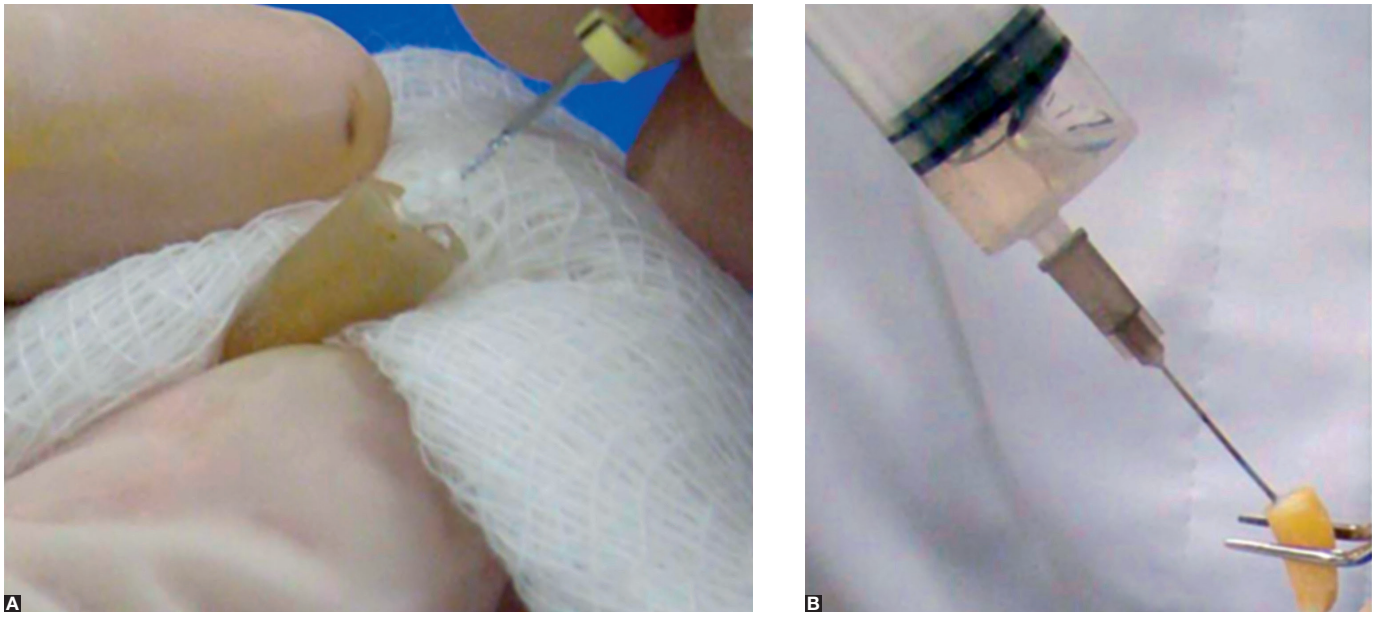

Figure 4. A) Insertion of calcium hydroxide paste, in toothpaste consistence, in roots by K-files #25. **B**) Insertion of others test substances in root canals according to each group.

The propylene glycol (Biológica Comércio e Manipulação de Medicamentos Ltda., Cuiabá, Brazil), copaiba oil (Flores e Ervas Produtos Naturais Ltda., Piracicaba, Brazil), extract of propolis (Flores e Ervas Produtos Naturais Ltda., Piracicaba, Brazil) and chlorhexidine gel 2% (Biológica Comércio e Manipulação de Medicamentos Ltda., Cuiabá, Brazil) were removed from the respective recipients by disposable syringes and inserted in the root canals of each test group (Fig 4B). The roots in the control groups did not receive microorganisms inoculation and no intracanal dressing.

A small sterile cotton pellet was inserted in the root canal entrance and again immersed in the BHI broth, taken to the incubator at 37° C for 7 days. After this period, the medications were removed from the root canals by K-files #25 (Maillefer Dentsply, Switzerland) and abundantly irrigated with 10 mL of sodium thiosulfate 10%. In continuous act, the roots were externally dried with gauze, internally with sterilized absorbent paper cones #50 (Dentsply, Petrópolis, Brazil) and taken in BHI broth at 37° C for 24 hours. The test tubes were analyzed by a unique calibrated examinator for the culture medium ranking, indicating the contamination presence.

Results

The results obtained in relation to the effectiveness of all of the substances were not efficient to the bacterial control, described in the Table 2.

Table 2. Values of presence or absence of culture medium turbidity.

Groups	
Copaiba oil	+ + +
Chlorhexidine gluconate gel 2%	+ + +
Extract of propolis	+ + +
Calcium hydroxide + propylene glycol	+ + +
Positive control (propylene glycol)	+ + +
Negative control	– – –

Discussion

Rests of pulpar tissues, bacteria and dentinal debris can persist the irregularities of the root canal system, even after the use of intracanal dressing, avoiding the endodontic treatment success.[1,2,3,12] For this study, the *Enterococcus faecalis* was selected in function of its microbiological role in the persistent infection processes in endodontic treatments.[11] Many methods are used to evaluate the antimicrobial activity of the intracanal dressing, and so *in vitro* test has the advantage of easy execution and fast in collecting the results, free of factors that can influence the results, inherent to *in vivo* studies.[4-8,11,13] The BHI culture medium was used for the evaluation because of being rich in appropriated nutrients to cultivate the *E. faecalis*.[22]

Classically, the calcium hydroxide was used to compare the new intracanal dressing substances in reason of been a consensus in its purpose in Endodontics.[12,13] This medication acts in the cellular wall of bacteria, causing damage to bacterial cytoplasmic membrane, protein denature and damage to DNA.[13,23] At same time, by the methodology used in this experiment, should be considered the buffer action of dentin that interferes particularly in the calcium hydroxide antibacterial ability.[24]

Comparatively, in this study, chlrohexidine 2% presented similar antibacterial activity to calcium hydroxide. The antibacterial effect of calcium hydroxide increases with the time, possibly as a result of the slow dissolution of the paste and respective effective diffusion of the hydroxyl ions through the dentin.[12,13] These findings are in agreement to others studies.[12,13,15]

The antimicrobial activity of propolis has been investigate in others studies by the dilution in broth and diffusion tests in agar; however, few are done in the inner of the root canal, environment that neutralize the antibacterial effects of the disinfectants.[7,15,24] In this study, the antibacterial effect of the propolis was similar to the others test substances. The mechanism of action that happen the antibacterial effect of this substance is related to the presence of flavonoids, many esters of caffeic acid and galagine (3,5,7-trihidroxiflavone) and its bioautograme components.[25] These findings are consonant to Oncang et al,[26] however different to others studies in that the extract of propolis presented greater antibacterial effect than the calcium hydroxide.[25,27,28]

Copaiba oil, as related in the literature, presents antibacterial activity against to gram positive bacteria, as it is *E. faecalis*.[21] The substance acts directly in the cellular wall of the bacteria, breaking and releasing cytoplasmic components, with consequent reduction of cell volume, thus proving its effectiveness.[21]

Conclusion

Within the limitations of the method proposed in this study, it was concluded that the tested drug substances were not effective against *E. faecalis*.

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