GABRIELY CRISTINNI REZENDE

AVALIAÇÃO DA AÇÃO ANTIMICROBIANA DOS CIMENTOS ENDODÔNTICOS PÓS PRESA, APÓS O USO DE HIDRÓXIDO DE CÁLCIO SOBRE BIOFILME DE ENTEROCOCCUS FAECALIS

Tese apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP como parte dos requisitos para obtenção do título de Doutora em Endodontia.

Orientador: Prof. Adj. Rogério Castilho Jacinto
Co-Orientadora: Profa. Dra. Carolina Simonetti Lodi

Araçatuba – SP
2019

Dissertação elaborada de acordo com a Instrução Normativa N.º014-CPPGCO, de 14 de março de 2013.

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

R467a

Rezende, Gabriely Cristinni.
Avaliação da ação antimicrobiana dos cimentos endodônticos pós presa, após o uso de hidróxido de cálcio sobre biofilme de Enterococcus faecalis / Gabriely Cristinni Rezende. - Araçatuba, 2019
94 f. : il. ; tab.

Tese (Doutorado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. Rogério Castilho Jacinto
Coorientador: Profa. Carolina Simonetti Lodi

1. Endodontia 2. Cimentos dentários 3. Hidróxido de cálcio 4. Enterococcus faecalis I. T.

Black D24
CDD 617.67

Claudio Hideo Matsumoto – CRB-8/5550

Dados Curriculares

GABRIELY CRISTINNI REZENDE

Nascimento	05 de setembro de 1990 Ilha Solteira/SP
Filiação	Eli Tamar Paulon Rezende Valter Pereira Rezende
2008-2012	Curso de Graduação em Odontologia – Fundação Municipal de Educação e Cultura/SP FUNEC
2012-2014	Curso de Especialização em Endodontia – Fundação Municipal de Educação e Cultura/SP FUNEC
2013-2015	Curso de Pós-Graduação em Endodontia, nível de Mestrado – Faculdade de Odontologia de Araçatuba – Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP
2015-2019	Curso de Pós-Graduação em Endodontia, nível de Doutorado – Faculdade de Odontologia de Araçatuba – Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP
2017 – 2019	Professora Substituta em Endodontia, Fisiologia, Trabalho de Conclusão de Curso e Clinicas Integradas – Centro Universitário de Santa Fé do Sul.

Dedicatória

Dedico esse trabalho, aos meus pais Eli Tamar e Valter, ao meu Irmão Cayo, e ao meu marido Juarez Humberto por serem meu alicerce durante a realização dos meus sonhos. Amo vocês!!!

Agradecimentos Especiais

À Deus

Por todos os sonhos realizados

À minha família e marido

Por todo carinho e dedicação

Ao Prof. Rogério Castilho Jacinto

Pela chance, confiança, orientação e pelos
conhecimentos repassados durante todo o doutorado.

A Profª Carolina Simonetti Lodi

Pela oportunidade, confiança, paciência, atenção, disposição, e
por todos os conhecimentos divididos durante todo o
desenvolvimento do trabalho.

À Faculdade de Odontologia de Araçatuba, da Universidade Estadual Paulista “Júlio de Mesquita filho” – UNESP, na pessoa da professora Dr. Prof. Tit. Wilson Roberto Poi, digníssima diretor.

Aos docentes da disciplina de Endodontia da Faculdade de Odontologia de Araçatuba – UNESP, Prof. Dr. Luciano Tavares Ângelo Cintra, Prof. Dr. João Eduardo Gomes Filho, Prof. Dr. Elói Dezan Junior e Prof. Dr. Gustavo Sivieri pelo aprendizado, apoio e contribuição na minha formação profissional!

Aos docentes da disciplina de Saúde Bucal da Criança da Faculdade de Odontologia de Araçatuba – UNESP, Profª. Ass. Dra. Cristiane Duque e Prof. Adj. Alberto Carlos Botazzo Delbem, pelo aprendizado, apoio e contribuição nos trabalhos desenvolvidos durante o mestrado e por abrirem as portas do laboratório do Departamento de Odontologia Infantil e Social!

Aos professores da Faculdade Integradas de Santa Fé do Sul, em especial ao Prof. Dr. Arnaldo e Prof. Guilherme, por todo apoio, atenção, e todos os conhecimentos passados durante a graduação, especialização e colegas de trabalho. Muitíssimo obrigado!!!

Aos Prof. Dr. João Eduardo Gomes Filho e Profª. Drª. Cristiane Duque, pelo apoio, auxílio para que esse trabalho acontecesse. Muito Obrigada!

Aos meus amigos de Pós Graduação da Endodontia da FOA/UNESP, Loiane, India, Amanda, Carol, Francine, Camila e Carlos pela convivência, amizade e apoio!

Aos meus colegas da Pós Graduação em Saúde Bucal da Criança da FOA/UNESP, Mariana, Thayse, Jaqueline Canova e Carla pela convivência e apoios.

Às amigas India e Loiane pela amizade, paciência, apoio, carinho e pela enorme ajuda durante o desenvolvimento desse trabalho. Muito obrigada, Meninas !

Resumo

Rezende, GC. Avaliação da ação antimicrobiana dos cimentos endodônticos pós presa, após o uso de hidróxido de cálcio sobre biofilme de *Enterococcus faecalis*. Araçatuba, 2019. 44p. Tese (Doutorado em Endodontia) – Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Resumo

O presente estudo teve como objetivo avaliar a atividade antimicrobiana dos cimentos endodônticos, com ou sem associação do uso prévio de hidróxido de cálcio em um modelo *"in vitro"* de biofilme. Espécimes de dentina bovina (240) foram colocadas e deixas em contato direto com inoculo de *E. faecalis* (ATCC 51299) por 14 dias, para induzir a formação do biofilme. Em seguida, metade das espécimes foram incubadas (37^0C e 5% CO^2) em contato com um dos seguintes cimentos: AH Plus, Acroseal e Sealapex por 2, 7 e 14 dias, e a outra metade foi tratada com solução de hidróxido de cálcio por 14 dias e incubada em contato com os cimentos AH Plus, Acroseal e Sealapex por 2, 7 e 14 dias. Cada grupo continha um n = 8. Após cada período experimental, as amostras foram agitadas e as suspensões formadas foram diluídas em série e triplamente plaqueadas em ágar m-Enterococcus. As unidades formadoras de colônias foram contadas, e os dados foram analisados estatisticamente usando os testes one-way ANOVA, Shapiro-Wilk e Kruskal-Wallis ($p < 0,05$) para determinar o potencial antimicrobiano. Foi observada diferença estatisticamente significante entre os grupos com e sem o tratamento com Hidróxido de Cálcio, para todos os cimentos avaliados. Entretanto, nenhum dos cimentos testados foi capaz de eliminar completamente o biofilme. Ao comparar os cimentos, Sealapex reduziu *E. faecalis* após 7 dias, enquanto AH Plus e Acroseal mostraram atividade antimicrobiana apenas no 14º dia experimental. Em conclusão, o uso prévio de hidróxido de cálcio ajudou a diminuir o biofilme de *Enterococcus faecalis* dos cimentos estudados em todos os tempos experimentais.

Palavras chaves: Endodontia, Cimentos dentários, Hidróxido de Cálcio, *Enterococcus faecalis*.

Abstract

Rezende, GC. Evaluation of the antimicrobial action of endodontic sealers after setting, after use of calcium hydroxide on *Enterococcus faecalis* biofilm. Araçatuba, 2019. 44p. Tese (Doctor in Endodontics) – Dental School of Araçatuba, São Paulo State University "Júlio de Mesquita Filho".

Abstract

The present study aimed at evaluating the antimicrobial activity of endodontic sealers, with or without prior use association of calcium hydroxide in an in vitro biofilm model. Bovine dentin specimens (240) were placed and left in contact with inoculum of *E. faecalis* (ATCC 51299) for 14 days, to induce biofilm formation. Then, half of the specimens were incubated (37^0C and 5% CO2) in contact with one of the following sealers AH Plus, Acroseal and Sealapex for 2, 7 and 14 days, and the other half were treated with calcium hydroxide solution for 14 days, and then incubated in contact with the sealers AH Plus, Acroseal and Sealapex for 2, 7 and 14 days. Each group comprised a n=8. After each experimental time the samples were agitated, and the suspensions formed were serially diluted, and triple plated onto m-Enterococcus agar. Colony forming units were counted, and the data were statistically analyzed using ANOVA, Shapiro-Wilk and Kruskal-Wallis one-way tests ($p<0.05$) to determine antimicrobial potential. A statistically significant difference was observed between the groups with and without the treatment with Calcium Hydroxide, for all sealers evaluated. However, neither of the sealers tested were able to completely eliminate the biofilm. When comparing the sealers, Sealapex reduced *E. faecalis* after 7 days, while AH Plus and Acroseal showed antimicrobial activity only on the 14th experimental day. In conclusion, previous use of calcium hydroxide helped to decrease *Enterococcus faecalis* biofilm of the sealers studied in all experimental times.

Key words: Endodontics, Dental cements, Calcium hydroxide, *Enterococcus faecalis*.

Lista de Abreviações

Lista de Abreviações

ADT – Agar Difussion Test
CFU - Colony-forming Units
DCT – Direct contact Test

Lista de Tabelas

Lista de Tabelas

Sumário

Artigo

Evaluation of the antimicrobial action of endodontic sealers after setting, after use of calcium hydroxide on *Enterococcus faecalis* biofilm

Abstract

The present study aimed at evaluating the antimicrobial activity of endodontic sealers, with or without prior use association of calcium hydroxide in an in vitro biofilm model. Bovine dentin specimens (240) were placed and left in contact with inoculum of *E. faecalis* (ATCC 51299) for 14 days, to induce biofilm formation. Then, half of the specimens were incubated (37^0C and 5% CO2) in contact with one of the following sealers AH Plus, Acroseal and Sealapex for 2, 7 and 14 days, and the other half were treated with calcium hydroxide solution for 14 days, and then incubated in contact with the sealers AH Plus, Acroseal and Sealapex for 2, 7 and 14 days. Each group comprised a n=8. After each experimental time the samples were agitated, and the suspensions formed were serially diluted, and triple plated onto m-Enterococcus agar. Colony forming units were counted, and the data were statistically analyzed using ANOVA, Shapiro-Wilk and Kruskal-Wallis one-way tests ($p<0.05$) to determine antimicrobial potential. A statistically significant difference was observed between the groups with and without the treatment with Calcium Hydroxide, for all sealers evaluated. However, neither of the sealers tested were able to completely eliminate the biofilm. When comparing the sealers, Sealapex reduced *E. faecalis* after 7 days, while AH Plus and Acroseal showed antimicrobial activity only on the 14th experimental day. In conclusion, previous use of calcium hydroxide helped to decrease *Enterococcus faecalis* biofilm of the sealers studied in all experimental times.

Key Words: Endodontics, Dental cements, Calcium hydroxide, *Enterococcus faecalis.*

Introduction

The persistence of bacterial infections after root canal treatment has been shown to induce or maintain chronic apical lesions.[1,2] Biomechanical preparation of the root canal acts especially on microorganisms located in the lumen of the root canal,[3] therefore, the local application of antibacterial root canal dressings may contribute to reduce bacteria located in ramifications of the root canal system.[4,5,6]

Calcium hydroxide has been used commonly as intracanal medication in endodontic treatment due to its biological properties, such as biocompatibility and capacity of altering the microbial enzymatic metabolism by promoting an highly alkaline environment.[7] Besides revealed that the use of intracanal medication with calcium hydroxide for 14 days resulted in improvement of endotoxin removal from primarily infected root canals, which confirm the importance of using an interappointment dressing to enhance the endotoxin-detoxifying effects of the chemomechanical preparation.[8] However, some microorganisms such as *Enterococcus faecalis* might resist the action of calcium hydroxide-based medications.[9,10,11,12]

E. faecalis is a Gram-positive facultative anaerobic cocci commonly found in cultures from failed root canal therapy.[13] This bacteria is capable of surviving extreme conditions such as heat (60 °C for 30 min); both acidic and alkaline pH (from 3.5 to 11); and high salt concentrations (6.5% NaCl).[14,15] It can also grow within infected root canals as biofilms attached to the canal walls even with limited carbohydrate supply;[16] and might survive starvation for 6 months, using low levels of serum for growth.[17] These characteristics favor the survival of this species to endodontic therapy, leading to

posttreatment apical periodontitis, being, therefore, frequently detected in persistent endodontic infections.[18,19,20]

The obturation of root canals with endodontic sealers with antimicrobial activity can help reducing the number of bacterial cells that have resisted the endodontic biochemical preparation and intracanal medication.[21,22] A variety of endodontic sealers are available in the market which include calcium hydroxide based sealers and resin-epoxy based sealers. The antimicrobial effect of these sealers depends on their chemical composition.[23]

Sealapex and Acroseal are calcium hydroxide-based sealers, which showed antimicrobial activity against *Enterococcus faecalis* in several studies.[24,25] Sealers containing calcium hydroxide might be used to alkalinize the environment, which happens due to dissociation of $Ca(OH)_2$ into calcium and hydroxyl ions, leading to an unfavorable environment for bacterial proliferation.[26]Moreover, these sealers reduce the gaseous source of anaerobic bacteria because calcium ions can react with carbon dioxide.[27] AH Plus is an epoxy resin-based sealer that demonstrated antimicrobial action against *Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus mutans*.[28]

Several studies have evaluated the antimicrobial activity of endodontic sealers, ;[22,24,25,28,29] however, the association of intracanal medication with calcium hydroxide in the antibiofilm activity of the endodontic sealers has not been evaluated yet. Therefore, the aim of this study was to evaluate, *in vitro*, the antimicrobial activity of endodontic sealers Acroseal, AH Plus and Sealapex, after the use of calcium hydroxide against biofilms of *E. faecalis*. The null hypothesis was that the association of calcium

hydroxide, as intracanal medication, with the endodontic sealers does not reduce *Enterococcus faecalis* biofilm.

Materials and methods

Sealer samples

The sealers used in the present study were: Acroseal (Septodont, Saint-Maur-des-Fossés, France), Sealapex (Sybron Kerr Co, Romulus, USA), and AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany). Sealer samples were prepared according to the manufacturers' recommendations, inserted into sterile silicone molds measuring 7 mm x 1 mm (internal diameter x thickness) in a laminar flow chamber (Veco Bioseg 12 Ltda., Campinas, Brazil) and kept at 37°C in controlled humidity for 56 hours. Calcium hydroxide paste was prepared using 1g of Calcium hydroxide PA (Biodinâmica Química & Farmacêutica LTDA, Ibiporã, Brazil) and 3ml sterile deionized water, and then weighed into falcon tubes which were added sterile distilled water at a ratio of 0.1g of the paste calcium hydroxide to 1 mL of sterile deionized water (0,1g/1 mL), and kept at 37°C for 24h.

Analysis of antibiofilm activity

Bovine incisors with completely formed roots were used as a substrate for biofilm development. The roots were sectioned into blocks measuring 4.0 mm x 4.0 mm x 1.5 mm (width x length x thickness) using a diamond disc at low speed, under abundant irrigation. The resulting blocks (240) were immersed in 17% EDTA for 3 minutes to remove the smear layer, and then placed in a test tube containing distilled water and sterilized by autoclaving at 121°C for 20 min.

The microbiological procedures and manipulation of the sterilized dentine blocks were carried out in a laminar flow chamber (Veco Bioseg 12 Ltda., Campinas, Brazil). A standard strain of *E. faecalis* (ATCC 51299) was used for biofilm formation. The microorganism was reactivated in 20 mL sterile Brain Heart Infusion (BHI) Agar (Difco Laboratories Inc., Detroit, USA) and kept at 37°C for 24 hours. The colonies were inoculated into 5.0 mL sterile brain heart infusion broth (Difco Laboratories Inc., Detroit, USA) and kept at 37°C overnight, after which the medium optical density was measured with a spectrophotometer (BioTek Instruments, Winooski, USA) set at 550 nm wavelength. The optical density was adjusted to 0.06 [approximately 9 x 10^7 colony-forming units per mL (CFU/mL)].

The bovine dentine blocks were placed in 24-well cell culture plates and each well received 200 µL of adjusted inoculum plus 1.8 mL of sterile BHI medium. The cell culture plates with the submerged bovine dentine blocks were kept in 5% CO_2 (Ultra Safe, HF212-UV) at 37°C for 14 days.[25,30] During this period, BHI medium of each well was changed completely every 48 h without adding new microorganisms.In the 14th day, the bovine dentine blocks was washed with distilled water for removal of planktonic cells.

The experiment was realized in two phases, in the 1st phase 1 mL of calcium hydroxide solution was added to 96 block in a new 24-well cell culture plates, which was kept in 5% CO_2 for 14 days at 37ºC. Other eight blocks were used for each material (calcium hydroxide – 1st phase, Acroseal, AH Plus and Sealapex) and each experimental point (2, 7 and 14 days). Thus, 1 mL of calcium hydroxide solution (Group calcium hydroxide – 1st phase) or endodontic sealer (Group Acroseal, Group AH Plus and Group Sealapex) sample was positioned over one of the dentine blocks containing biofilm. The dentine blocks/material samples were placed in new 24-well cell culture plates and kept in 5% CO2 for 2, 7 and 14 days at 37°C. Eight discs of each material were used for each period, and for each material, and eight dentin blocks with formed

biofilm that were not placed in contact with any material were used as negative controls (Group Control).

In the 2nd phase, after 14 days the 96 blocks that remained in direct contact with calcium hydroxide solution, were washed and each endodontic sealer sample was positioned over one of the dentine blocks. The dentine blocks/material samples were placed in new 24-well cell culture plates and kept in 5% CO_2 for 2, 7 and 14 days at 37°C. Even as the 1st phase, eight discs of each material (Group Acroseal + HC, Group AH Plus + CH and Sealapex + CH) were used for each period and material. Eight dentin blocks with formed biofilm that were not placed in contact with any material were used as negative controls (Group Control), and eight dentin blocks that were submitted to treatment with calcium hydroxide without sealers were used as control calcium hydroxide substantivity for each period of evaluation (Group Calcium hydroxide – 2nd phase). Previous tests showed that dentine block/material samples have to be hydrated with 20 µL of saline every day to simulate the root canal environment as well as possible.[25]

After the respective contact periods elapsed (2, 7 or 14 days), the endodontic sealer discs or calcium hydroxide solution (Group calcium hydroxide – 1st phase) were removed, and the dentine blocks containing the remaining biofilm, including those belonging to the control group, were stored individually in test tubes containing 1 mL of sterile saline. The tubes were agitated with a sonicator (Misonix Inc., Fransingdale, USA) for 30 s at 40 W to disrupt the biofilm.

The suspensions of *E. faecalis* were serial diluted (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6}), and 10 µL aliquots of each suspension were used for inoculation in Petri dishes containing M-Enterococcus agar medium (Acumedia – Neogen Corporation, Lansing, USA); the dishes were then incubated in 5% CO_2 at 37°C for 48 h in triplicate. The readings for each Petri dish were performed on areas of bacterial growth, where the dilutions generated were between 3 and 30 colonies. The number of CFU/mL was calculated for each group.

Statistical Analysis

Statistical analysis was performed using Sigma Plot 12.0 (Systat Software Inc., San Jose, USA). The median for the parameter measured (CFUs in the biofilm) were calculated for each group. The data were analyzed using a single-factor ANOVA model and Shapiro-Wilk, since they were not distributed normally, and the variances were not equal. The Kruskal-Wallis one-way test was also used. The level of significance was set at 5%.

Results

Table 1 shows the median CFU/mL^{-1} in the different groups and periods. Comparison between the groups revealed that the use of calcium hydroxide previous to contact with endodontic sealers reduced *E. faecalis* in all periods evaluated. When compare the sealers, Sealapex reduced *E. faecalis* after 7 and 14 days, while AH Plus and Acroseal reduce the CFU count only after 14 days. Comparison between periods revealed that all the sealers evaluated have reduced *E. faecalis* CFU after 14th days of direct contact. Comparison between the groups calcium hydroxide and calcium hydroxide control showed that after remove the dressing, there was no microbial reduction . However, *E. faecalis* biofilm was not completely eliminated in any group.

Discussion

Contact of the sealers with the biofilm after treatment with calcium hydroxide showed a larger antimicrobial activity in comparison to sealers applied over the biofilm without previous treatment with calcium hydroxide. The sealers Sealapex, AH Plus and

Acroseal with treatment calcium hydroxide showed reduction, comparing the sealers after 2 and 14 days of direct contact with the biofilm. Therefore, the null hypothesis was rejected, because previous contact of the biofilm with calcium hydroxide solution showed superior antimicrobial activity, when compared with sealers without previous use calcium hydroxide of the all sealers tested.

The aim of this study was to evaluate the antibacterial activity of endodontic sealers, after using calcium hydroxide through a Direct Contact Test with *E. faecalis* biofilm. The Direct Contact Test (DCT) was adopted because direct contact of the materials with the biofilm grown on dentin blocks avoids false positive results attributed to physical entombment of bacteria in root canals and dentinal tubules.[25,30,31] *E. faecalis* was chosen due to its prevalence in teeth with failure of the endodontic treatment.[32] The presence of a large number of CFU in the control group at all periods evaluated confirms the methodology effectiveness in biofilm formation in dentin tubules.[25,30,31]

The endodontic treatement in a single-visit has shown a success rate of 46% versus 74% when an intracanal medication with calcium hydroxide is used between appointments.[33] Silveira et al.[33] showed intensely inflamed periradicular tissues, areas of active root resorption, bacteria on the root canal walls and sometimes within dentinal tubules, in failed cases of single-visit treatments. In contrast, when an intracanal medication with calcium hydroxide was used, they observed periradicular tissues free of inflammation or with mild inflammation, with evidence of healing, and areas of root resorption covered by cementum, indicating a repair and resorption ceased, and bacteria-free root canals.[33] In the present study, calcium hydroxide was associated with distilled water, and showed reduction of bacterial cells after 14 days when compared with the control group, but the biofilm was not completely eliminated, which is supported by previous studies.[34] However, after removal the dressing the count CFU

maintained, demonstrating that calcium hydroxide did not continue acting on the biofilm, i.e., calcium hydroxide has no substantivity.[35,36]

This study verified that the use of calcium hydroxide previous to endodontic sealers with antimicrobial action can further reduce the number of *E .faecalis*. Sealapex with use of calcium hydroxide reduced of CFU/ml after 2 days of direct contact, reaching the highest reduction after 14 days, when compared with Sealapex group. Acroseal and AH Plus with calcium hydroxide also demonstrated reduction in all periods evaluated (2, 7 and 14 days), all of them with better results after 14 days of direct contact with biofilm.

In the present study, the best antimicrobial activity occurred with Sealapex than with AH Plus and Acroseal after use calcium hydroxide after 7 days. This difference is probably associated with the solubility of Sealapex, leading to dissociation of Ca++ and OH−,[37] on the other hand epoxy resin-based cements are highly insoluble.[38] Assessing the effect of root canal dressings in the antimicrobial activity showed reduction in *E. faecalis* biofilm, mainly associated with Sealapex ($P<0.05$). The dissociation of calcium hydroxide into calcium and hydroxide ions is responsible for increasing the pH of the root canal system, making it unfavorable for bacterial growth.[21] The superiority of Sealapex over different root canal sealers in antimicrobial activity has been reported in other studies.[25]The use of sealers with antimicrobial activity is an important auxiliary factor for the elimination of remaining bacteria in the root canal system after biomechanical preparation and the application of a root canal dressing. [6,39]

Conclusion

The use previous calcium hydroxide medication improves the antibiofilm activity of endodontic sealers, especially when associated with Sealapex. Sealapex showed better results than Acroseal and AH Plus. Besides Sealapex showed antibiofilm activity after 7 regardless of previous contact with the medication , while Acroseal and AH Plus only started acting against biofilm after 14 days.

References

1. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol. 1990 Apr;6(2):73-7.
2. Leonardo MR, Almeida WA, Silva LAB, Utrila LS. Histopathological observations of periapical repair in teeth with radiolucent areas submitted to two different methods of root canal treatment. J Endod. 1995 Mar;21(3):137-41.
3. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985 Jan;18(1):35-40.
4. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol. 1987 Apr;3(2):86-90.
5. Sundqvist G. Ecology of the root canal flora. J Endod. 1992 Sep;18(9):427-30.
6. Leonardo MR, Almeida WA, Ito IY, Silva LAB. Radiographic and microbiological evaluation of posttreatment apical and periapical repair of dog's teeth with experimentally induced chronic lesion. Oral Surg Oral Med Oral Pathol. 1994 Aug;78(2):232-8.
7. Estrela C, Sydney GB, Bammann LL, Felippe Júnior O. Mechanism of the action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 1995;6(2):85-90.
8. Xavier AC, Martinho FC, Chung A, Oliveira LD, Jorge AO, Valera MC, Carvalho CA. One-visit versus two-visit root canal treatment: effectiveness in the removal of endotoxins and cultivable bacteria. J Endod. 2013 Aug;39(8):959-64.
9. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinl tubules. Endod Dent Traumatol. 1990 Aug;6(4):142-9.
10. Waltimo TMT, Orstavik D, Sirén EK, Haapasalo MP. In vitro susceptibility of Candida albicans to four disinfectants and their combinations. Int Endod J. 1999 Nov;32(6):421-9.
11. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. Int Endod J. 2002 Mar;35(3):221-8.

12. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by Enterococcus faecalis. J Endod. 2005 Dec;31(12):867-72.
13. Fouad AF, Zerella J, Barry J, Spanberg LS. Molecular detection of enterococcus species in root canals of therapy-resistant endodon-tic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005 Jan;99(1):112-8.
14. Boutibonnes P, Giard JC, Hartke A, Thammavongs B, Auffray Y. Characterization of the heat shock response in Enterococcus faecalis. Antonie Van Leeuwenhoek. 1993;64(1):47-55.
15. Flahaut S, Hartke A, Giard JC, Auffray Y. Alkaline stress response in Enterococcus faecalis: adaptation, cross-protection, and changes in protein synthesis. Appl Environ Microbiol. 1997 Feb;63(2):812-4.
16. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic enterococcus faecalis J Endod. 2007 Jul;33(7):815-8. Epub 2007 Apr 16.
17. Richards D, Davies JK, Figdor D. Starvation survival and recovery in serum of Candida albicans compared with Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Jul;110(1):125-30.
18. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006 Feb;32(2):93-8.
19. Zhang C, Du J, Peng Z. Correlation between Enterococcus faecalis and persistent intraradicular infection compared with primary intraradicular infection: a systematic review J Endod. 2015 Aug;41(8):1207-13.
20. Persoon IF, Crielaard W, Ozok AR. Prevalence and nature of fungi in root canal infections: a systematic review and meta-analysis. Int Endod J. 2017 Nov;50(11):1055-66.
21. Berbert FL1, Leonardo MR, Silva LA, Tanomaru Filho M, Bramante CM. Influence of root canal dressings and sealers on repair of apical periodontitis after endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Feb;93(2):184-9.
22. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009 Jul;35(7):1051-5
23. Teixeira AB, Vidal CL, Castro DT, Olveira-Santos C, Schiavon MA, Reis AC. Incorporating antimicrobial nanomaterial and its effect on the antimicrobial activity, flow and radiopacity of endodontic sealers. Eur Endod J. 2017;16:1–8.
24. Dalmia S , Gaikwad A, Samuel R, Aer A, Gulve M, Kolhe S . Antimicrobial Efficacy of Different Endodontic Sealers against Enterococcus faecalis: An In vitro Study. J Int Soc Prev Community Dent. 2018 Mar-Apr; 8(2):104-109.
25. Rezende GC, Massunari L, Queiroz IO, Gomes Filho JE, Jacinto RC, Lodi CS, Dezan Junior E. Antimicrobial action of calcium hydroxide-based endodontic sealers after setting, against E. faecalis biofilm. Braz Oral Res. 2016;30. pii: S1806-83242016000100228.
26. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod. 2003 Apr;29(4):257-8.

27. Eldeniz AU, Erdemir A, Kurtoglu F, Esener T. Evaluation of pH and calcium ion release of Acroseal sealer in comparison with Apexit and Sealapex sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Mar;103(3):e86-91.
28. Kapralos V1, Koutroulis A2, Ørstavik D3, Sunde PT3, Rukke HV2. Antibacterial Activity of Endodontic Sealers against Planktonic Bacteria and Bacteria in Biofilms. J Endod. 2018 Jan;44(1):149-154.
29. Pinheiro ET, Gomes BPFA, Ferraz CCR, Sousa ELR, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J. 2003 Jan;36(1):1-11.
30. Faria-Júnior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J. 2013 Aug;46(8):755-62.
31. Guerreiro-Tanomaru JM, de Faria-Júnior NB, Duarte MA, Ordinola-Zapata R, Graeff MS, Tanomaru-Filho M. Comparative analysis of Enterococcus faecalis biofilm formation on different substrates. J Endod. 2013 Mar;39(3):346-50.
32. Duarte MA, Ordinola-Zapata R, Bernardes RA, et al. Influence of calcium hydroxide association on the physical properties of AH Plus. J Endod. 2010 Jun;36(6):1048-51.
33. Silveira AM, Lopes HP, Siqueira JF Jr, Macedo SB, Consolaro A. Periradicular repair after two-visit endodontic treatment using two different intracanal medications compared to single-visit endodontic treatment. Braz Dent J. 2007;18(4):299-304.
34. Sangalli J, Júnior EGJ, Bueno CRE, Jacinto RC, Sivieri-Araújo G, Filho JEG, Cintra LT, Júnior ED. Antimicrobial activity of Psidium cattleianum associated with calcium hydroxide against Enterococcus faecalis and Candida albicans: an in vitro study. Clin Oral Investig. 2018 Jul;22(6):2273-9.
35. Messer HH, Chen RS. The duration of effectiveness of root canal medicaments. J Endod. 1984 Jun;10(6):240-5.
36. Lenet BJ, Komorowski R, Wu XY, Huang J, Grad H, Lawrence HP, Friedman S. Antimicrobial substantivity of bovine root dentin exposed to different chlorhexidine delivery vehicles. J Endod. 2000 Nov;26(11):652-5.
37.Tagger M, Tagger E, Kfir A. Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. J Endod. 1988 Dec;14(12):588-91.
38. Orstavik D. Weight loss of endodontic sealers, cements and pastes in water. Scand J Dent Res. 1983 Aug;91(4):316-9.
39. M Tanomaru Filho, MR Leonardo, LAB Silva, LS. Utrilla. Effect of different root canal sealers on periapical repair of teeth with chronic periradicular periodontitis. Int Endod J. 1998 Mar;31(2):85-9.

Table 1. Median of CFU (CFU/ml^{-1}) in the different groups and periods

Material	2 DAYS	7 DAYS	14 DAYS
Acroseal	26,0 x 10^5 A.a	37,0 x 10^5 A,a	0,27 x 10^4 C,b
Acroseal + CH	2,1 x 10^5 B,a	7,1 x 10^5 B,a	0,04 x 10^5 D,b
AH Plus	35,0 x 10^5 A,a	37,0 x 10^5 A,a	0,27 x 10^5 C,b
AH Plus + CH	4,2 x 10^5 B,a	7,1 x 10^5 B,a	0,04 x 10^5 D,b
Sealapex	34,0 x 10^6 A,a	0,4 x 10^5 C,b	0,05 x 10^5 D,c
Sealapex + CH	2,7 x 10^5 B,a	0,04 x 10^5 D,b	0,005 x 10^5 E,c
Control	27,0 x 10^5 A,a	27,0 x 10^5 A,a	61,0 x 10^5 A,a
Calcium hydroxide – 1st Phase	17,0 x 10^5 A,a	49,0 x 10^5 A,a	3,4 x 10^5 B,b
Calcium hydroxide – 2nd Phase	6,3 x 10^5 B,a	3,0 x 10^5 B,a	4,1 x 10^5 B,a

*Different uppercase letters indicate a statistical difference (P <0.05) among the groups

*Different lowercase letters indicate a statistical difference (P<0.05) among the time periods

Anexos

Anexo A

Protocolo da Confecção dos Discos dos Cimentos

Preparo dos moldes: Colocar cerca de 20 arruelas de inox com diâmetro interno de 7mm na base da plastificadora a vácuo, e moldar as arruelas. Levar os moldes para esterilização em oxido de etileno em empresa especializada. Somente com os moldes em mão começar o experimento.

Preparo dos cimentos: - Estilizar uma placa de vidro, uma espátula, e um conjunto de placas petris de vidro para cada cimento, e gazes;

- Preparar os cimentos em fluxo laminar e colocar nos moldes sem tocar a parte interna dos moldes;

- Colocar os moldes nas placas Petris com gazes húmidas;

- Levar as placas para a estufa a 37º por 56h;

- Remover os discos do molde com o auxilio de uma pinça estéril em fluxo laminar.

Obs: as gazes devem ser humedecidas todos os dias até o final das 56h.

Anexo B

Protocolo do Ensaio de Biofilme em Blocos de Dentina

Preparo dos blocos: Cortar blocos 4x4mm da face proximal de incisivos bovinos na cortadeira;

Polir as duas faces do bloco em lixa de granulação 320, de forma que os blocos fiquem planos, sem ranhuras e com espessura entre 1,45mm e 1,55mm;

Esterilizar os blocos em tubo falcon com água destilada, por 20 min a 121°C.

Dia 1: Reativar o microrganismo em BHI agar (estriar 15µL da cultura estoque – Técnica do esgotamento) e incubar por 24h em estufa de CO_2 (5% CO_2 em 37°C).

Dia 2: Repicar o microrganismo da placa para o respectivo meio de cultura em caldo (5 UFC em 5mL) e incubar.

Dia 3: Preparar a placa de 24 poços com fundo plano (não tratada): colocar um bloco estéril em cada poço.

- Preparar o inóculo de acordo com a curva de crescimento: D.O. 0,06;

- Acrescentar 200µL do inóculo e 1800µL de BHI em cada poço, e incubar por 48h.

Dia 5: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 7: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 9: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 11: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 13: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 14: Preparar os cimentos de acordo com as recomendações do fabricante e colocar em moldes de 7,0 X 1,0 mm, colocar na estufa em placas Petri estéril com algodão umedecido por 56h;

Dia 15: Remover todo o meio com ponteira estéril, e acrescentar 2000µL de BHI fresco;

Dia 16: Preparar a solução de hidróxido de cálcio:

- A pasta de hidróxido de cálcio foi preparada com 1g de hidróxido de cálcio e 3mL de água destilada estéril;

- As pastas prontas foram colocadas em tubos de 15mL, pesadas e 1mL de água destilada estéril foi adicionada na proporção de 0,1g de pasta para 1mL de água;

- Os tubos foram deixados em base agitadora por 24horas antes do uso.

Dia 17: Remover os discos de cimentos dos moldes;

-Remover todo o meio com ponteira estéril, lavar cada bloco com 2mL de solução salina;

- Colocar os blocos em placas de 24 poços estéreis;

- Acrescentar os cimentos em metade dos blocos e hidratar cada poço com 20µL de solução salina;

*Os poços foram hidratados a cada 24h com 20µL de solução salina;

- Acrescentar 1mL de solução de hidróxido de cálcio na outra metade dos blocos;

Dia 19, 24 e 31: Remover toda solução de hidróxido de cálcio e cimentos dos grupos a serem avaliados;

Lavar os blocos com 2mL de solução salina;

Colocar os blocos em tubos falcon contendo 1mL de solução salina;

Sonicar cada tubo por 30 s a amplitude 60.

Agitar cada tubo por 30 s.

Diluir o conteúdo de cada tubo em 6 tubos de ensaio tipo eppendorf com 900µL de solução salina.

Plaquear em meio de cultura M-Enterococcus. (3 gotas de 10uL em cada quadrante)

Dia 21, 26 e 33: Contar as placas (3-30 colônias):

$$UFC = \frac{\text{Soma do número de colônias por gota}}{3} \times 10^2 \times 10^{\text{quadrante}}$$

Dia 28: Preparar os cimentos de acordo com as recomendações do fabricante e colocar em moldes de 7,0 X 1,0 mm, colocar na estufa em placas Petri estéril com algodão umedecido por 56h;

Dia 31: Remover os discos de cimentos dos moldes;

-Remover toda solução de hidróxido de cálcio com ponteira estéril, lavar cada bloco com 2mL de solução salina;

- Colocar os blocos em placas de 24 poços estéreis;

- Acrescentar os cimentos sobre os blocos e hidratar cada poço com 20µL de solução salina;

*Os poços foram hidratados a cada 24h com 20µL de solução salina;

Dia 33, 38 e 45: Remover os discos de cimentos;

Lavar os blocos com 2mL de solução salina;

Colocar os blocos em tubos falcon contendo 1mL de solução salina;

Sonicar cada tubo por 30 s a amplitude 60.

Agitar cada tubo por 30 s.

Diluir o conteúdo de cada tubo em 6 tubos de ensaio tipo eppendorf com 900µL de solução salina.

Plaquear em meio de cultura M-Enterococcus. (3 gotas de 10uL em cada quadrante)

Dia 35, 40 e 47: Contar as placas (3-30 colônias):

$$UFC = \frac{\text{Soma do número de colônias por gota}}{3} \times 10^2 \times 10^{\text{quadrante}}$$

Anexo C

Guide for Authors (Brazilian Oral Research)

Mission, scope, and submission policy

Brazilian Oral Research - BOR (online version ISSN 1807-3107) is the official publication of the Sociedade Brasileira de Pesquisa Odontológica - SBPqO (the Brazilian division of the International Association for Dental Research - IADR). The journal has an Impact Factor™ of 0.937 (Institute for Scientific Information - ISI), is peer-reviewed (double-blind system), and its mission is to disseminate and promote an information interchange concerning the several fields in dentistry research and/or related areas with gold open access.

BOR invites the submission of original and review manuscripts and papers in the following typology: Original Research (complete manuscript or Short Communication), Critical Review of Literature, Systematic Review (and Meta-Analysis) and Letters to the Editor. All submissions must be exclusive to.

Manuscripts and all corresponding documentation should be exclusively submitted through ScholarOne Manuscripts™ via the online submission link (http://mc04.manuscriptcentral.com/bor-scielo).

The evaluation process of manuscript's scientific content will only be initiated after meeting of all the requirements described in the present Instructions for Authors. Any manuscript that does not meet these requirements will be returned to the corresponding author for adaptations.

Important: Once having been accepted on their scientific merit, all manuscripts will be submitted for grammar and style revision as per the English language. Contact BOR by bor@sbpqo.org.br to get information about the recommended translation companies.The authors should forward the revised text with the enclosed revision certificate provided by the chosen editing company. Linguistic revisions performed by companies that do not provide the mentioned certificate will not be accepted. As an exception, this rule does not apply when one of the authors is a native English speaker.

Presentation of the manuscript

The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

All figures (including those in layouts/combinations) must be provided in individual and separate files, according to recommendations described under the specific topic.
Photographs, micrographs, and radiographs should be provided in TIFF format, according to the recommendations described under the specific topic.

Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format individually in separate files, according to the recommendations described under the specific topic.

Video files may be submitted as per the specifications, including the author's anonymity (for purposes of evaluation) and respect for the patient's rights.

Important: ScholarOne™ allows upload of a set of files up to 10 MB. In case the video file exceeds this size, it is possible to leave information about the link to access the video. The use of patients' initials, names, and/or registry numbers is prohibited in the reproduction of clinical documentation. The identification of patients is prohibited. An informed consent statement, signed by the patient, concerning the use of his/her

image should be provided by the author(s) when requested by BOR. The Copyright legislation in force must be respected and the source cited when the manuscript reproduces any previously published material (including texts, charts, tables, figures, or any other materials).

Title page (compulsory data)

This must indicate the specialty* or research field focused on in the manuscript.

*Anatomy; Basic Implantodontology and Biomaterials; Behavioral Sciences; Biochemistry; Cariology; Community Dental Health; Craniofacial Biology; Dental Materials; Dentistry; Endodontic Therapy; Forensic Dentistry; Geriatric Dentistry; Imaginology; Immunology; Implantodontology – Prosthetics; Implantodontology – Surgical; Infection Control; Microbiology; Mouth and Jaw Surgery; Occlusion; Oral Pathology; Orthodontics; Orthopedics; Pediatric Dentistry; Periodontics; Pharmacology; Physiology; Prosthesis; Pulp Biology; Social/Community Dentistry; Stomatology; Temporomandibular Joint Dysfunction.

Informative and concise title, limited to a maximum of 110 characters, including spaces.

Names of all authors written out in full, including respective telephone numbers and email addresses for correspondence. We recommend that authors collate the names present in the Cover Letter with the profile created in ScholarOne™, to avoid discrepancies. The participation of each author must be justified on a separate page, which should meet the authorship and co-authorship criteria adopted by the International Committee of Medical Journal Editors, available at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html.

Data of institutional/professional affiliation of all authors, including university (or other institution), college/program, department, city, state, and country, presented according to internal citation norms established by each author's institution. Verify that such affiliations are correctly entered in ScholarOne™.

Abstract: This should be presented as a single structured paragraph (but with no subdivisions into sections) containing the objective of the work, methodology, results, and conclusions. In the System if applicable, use the Special characters tool for special characters.

Keywords: Ranging from 3 (three) to 5 (five) main descriptors should be provided, chosen from the keywords registered at http://decs.bvs.br/ or http://www.nlm.nih.gov/mesh/MBrowser.html (no synonyms will be accepted).

Main Text

Introduction: This should present the relevance of the study, and its connection with other published works in the same line of research or field, identifying its limitations and possible biases. The objective of the study should be concisely presented at the end of this section.

Methodology: All the features of the material pertinent to the research subject should be provided (e.g., tissue samples or research subjects). The experimental, analytical, and statistical methods should be described in a concise manner, although in detail, sufficient to allow others to recreate the work. Data from manufacturers or suppliers of products, equipment, or software must be explicit when first mentioned in this section, as follows: manufacturer's name, city, and country. The computer programs and statistical methods must also be specified. Unless the objective of the work is to compare products or specific systems, the trade names of techniques, as well as

products, or scientific and clinical equipment should only be cited in the "Methodology" and "Acknowledgments" sections, according to each case. Generic names should be used in the remainder of the manuscript, including the title. Manuscripts containing radiographs, microradiographs, or SEM images, the following information must be included: radiation source, filters, and kV levels used. Manuscripts reporting studies on humans should include proof that the research was ethically conducted according to the Helsinki Declaration (World Medical Association, http://www.wma.net/en/30publications/10policies/b3/). The approval protocol number issued by an Institutional Ethics Committee must be cited. Observational studies should follow the STROBE guidelines (http://strobe-statement.org/), and the check list must be submitted. Clinical Trials must be reported according to the CONSORT Statement standard protocol (http://www.consort-statement.org/); systematic reviews and meta-analysis must follow the PRISMA (http://www.prisma-statement.org/), or Cochrane protocol (http://www.cochrane.org/).

Clinical Trials

Clinical Trials according to the CONSORT guidelines, available at www.consort-statement.org. The clinical trial registration number and the research registration name will be published along with the article.

Manuscripts reporting studies performed on animals must also include proof that the research was conducted in an ethical manner, and the approval protocol number issued by an Institutional Ethics Committee should be cited. In case the research contains a gene registration, before submission, the new gene sequences must be included in a public database, and the access number should be provided to BOR. The authors may use the following databases:

GenBank: http://www.ncbi.nlm.nih.gov/Genbank/submit

EMBL: http://www.ebi.ac.uk/embl/Submission/index.html

DDBJ: http://www.ddbj.nig.ac.jp

Manuscript submissions including microarray data must include the information recommended by the MIAME guidelines (Minimum Information About a Microarray Experiment: http://www.mged.org/index.html) and/or itemize how the experimental details were submitted to a publicly available database, such as:

ArrayExpress: http://www.ebi.ac.uk/arrayexpress/

GEO: http://www.ncbi.nlm.nih.gov/geo/

Results: These should be presented in the same order as the experiment was performed, as described under the "Methodology" section. The most significant results should be described. Text, tables, and figures should not be repetitive. Statistically relevant results should be presented with enclosed corresponding p values.

Tables: These must be numbered and cited consecutively in the main text, in Arabic numerals. Tables must be submitted separately from the text in DOC, DOCX, or RTF format.

Discussion: This must discuss the study results in relation to the work hypothesis and relevant literature. It should describe the similarities and differences of the study in relation to similar studies found in literature, and provide explanations for the possible differences found. It must also identify the study's limitations and make suggestions for future research.

Conclusions: These must be presented in a concise manner and be strictly based on the results obtained in the research. Detailing of results, including numerical values, etc., must not be repeated.

Acknowledgments: Contributions by colleagues (technical assistance, critical comments, etc.) must be given, and any bond between authors and companies must be revealed. This section must describe the research funding source(s), including the corresponding process numbers.

Plagiarism

BOR employs a plagiarism detection system. When you send your manuscript to the journal it may be analyzed-not merely for the repetition of names/affiliations, but rather the sentences or texts used.

References: Only publications from peer-reviewed journals will be accepted as references. Unfinished manuscripts, dissertations, theses, or abstracts presented in congresses will not be accepted as references. References to books should be avoided.

Reference citations must be identified in the text with superscript Arabic numerals. The complete reference list must be presented after the "Acknowledgments" section, and the references must be numbered and presented in Vancouver Style in compliance with the guidelines provided by the International Committee of Medical Journal Editors, as presented in Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.ncbi.nlm.nih.gov/books/NBK7256/). The journal titles should be abbreviated according to the List of Journals Indexed in Index Medicus (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The authors shall bear full responsibility for the accuracy of their references.

Spelling of scientific terms: When first mentioned in the main text, scientific names (binomials of microbiological, zoological, and botanical nomenclature) must be written out in full, as well as the names of chemical compounds and elements.

Units of measurement: These must be presented according to the International System of Units (http://www.bipm.org or http://www.inmetro.gov.br/consumidor/unidLegaisMed.asp).

Footnotes on the main text: These must be indicated by asterisks and restricted to the bare minimum.

Figures: Photographs, microradiographs, and radiographs must be at least 10 cm wide, have at least 500 dpi of resolution, and be provided in TIFF format. Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format. All the figures must be submitted individually in separate files (not inserted into the text file). Figures must be numbered and consecutively cited in the main text in Arabic numerals. Figure legends should be inserted together at the end of the text, after the references.

Characteristics and layouts of types of manuscripts

Original Research

Limited to 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 8 (eight) figures and 40 (forty) references will be accepted. The abstract can contain a maximum of 250 words.

Layout - Text Files

Title Page
Main text (30,000 characters including spaces)
Abstract: a maximum of 250 words
Keywords: 3 (three)-5 (five) main descriptors

Introduction
Methodology
Results
Discussion
Conclusion
Acknowledgments
Tables
References: maximum of 40 references
Figure legends
Layout - Graphic Files

Figures: a maximum of 8 (eight) figures, as described above.
Short Communication
Limited to 10,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 2 (two) figures and 12 (twelve) references will be allowed. The abstract can contain a maximum of 100 words.

Layout - Text Files

Title page
Main text (10,000 characters including spaces)
Abstract: a maximum of 100 words
Descriptors: 3 (three)-5 (five) main descriptors
Introduction
Methodology
Results
Discussion
Conclusion
Acknowledgments
Tables
References: a maximum of 12 references
Figure legends
Layout- Graphic Files

Figures: a maximum of 2 (two) figures, as described above.

Critical Review of Literature

The submission of this type of manuscript will be performed only by invitation of the BOR Publishing Commission. All manuscripts will be submitted to peer-review. This type of manuscript must have a descriptive and discursive content, focusing on a comprehensive presentation and discussion of important and innovative scientific issues, with a limit of 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). It must include a clear presentation of the scientific object, logical argumentation, a methodological and theoretical critical analysis of the studies, and a summarized conclusion. A maximum of 6 (six) figures and 50 (fifty) references is permitted. The abstract must contain a maximum of 250 words.

Layout- Text Files

Title page
Main text (30,000 characters including spaces)
Abstract: a maximum of 250 words
Keywords: 3 (three)-5 (five) main descriptors
Introduction
Methodology
Results
Discussion
Conclusion
Acknowledgments
Tables
References: maximum of 50 references
Figure legends
Layout - Graphic Files

Figures: a maximum of 6 (six) figures, as described above.

Systematic Review and Meta-Analysis

While summarizing the results of original studies, quantitative or qualitative, this type of manuscript should answer a specific question, with a limit of 30,000 characters, including spaces, and follow the Cochrane format and style (www.cochrane.org). The manuscript must report, in detail, the process of the search and retrieval of the original works, the selection criteria of the studies included in the review, and provide an abstract of the results obtained in the reviewed studies (with or without a meta-analysis approach). There is no limit to the number of references or figures. Tables and figures, if included, must present the features of the reviewed studies, the compared interventions, and the corresponding results, as well as those studies excluded from the review. Other tables and figures relevant to the review must be presented as previously described. The abstract can contain a maximum of 250 words.

Layout - Text Files

Title page
Main text (30,000 characters including spaces)
Abstract: a maximum of 250 words
Question formulation
Location of the studies
Critical Evaluation and Data Collection
Data analysis and presentation
Improvement
Review update
References: no limit on the number of references
Tables
Layout - Graphic Files

Figures: no limit on the number of figures
Letter to the Editor

Letters must include evidence to support an opinion of the author(s) about the scientific or editorial content of the BOR, and must be limited to 500 words. No figures or tables are permitted.

Copyright transfer agreement and responsibility statements

The manuscript submitted for publication must include the Copyright Transfer Agreement and the Responsibility Statements, available in the online system and mandatory.

CHECKLIST FOR INITIAL SUBMISSION

Title Page file (in DOC, DOCX, or RTF format).
Main text file (Main Document, manuscript), in DOC, DOCX, or RTF format.
Tables, in DOC, DOCX, or RTF format.
Declaration of interests and funding, submitted in a separate document and in a PDF format. (if applicable)
Justification for participation of each author, provided in a separate document and in a PDF format.
Photographs, microradiographs, and radiographs (10 cm minimum width, 500 dpi minimum resolution) in TIFF format. (http://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/)
Charts, drawings, layouts, and other vector illustrations in a PDF format.
Each figure should be submitted individually in separate files (not inserted in the text file).

Publication fees

Authors are not required to pay for the submission or review of articles.

EXAMPLES OF REFERENCES

Journals

Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, et al. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci. 2004 Aug;112(4):353-61.

Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics. 2005;115(2 Suppl):519-617. doi:10.1542/peds.2004-1441.

Usunoff KG, Itzev DE, Rolfs A, Schmitt O, Wree A. Nitric oxide synthase-containing neurons in the amygdaloid nuclear complex of the rat. Anat Embryol (Berl). 2006 Oct 27. Epub ahead of print. doi: 10.1007/s00429-006-0134-9

Walsh B, Steiner A, Pickering RM, Ward-Basu J. Economic evaluation of nurse led intermediate care versus standard care for post-acute medical patients: cost minimisation analysis of data from a randomised controlled trial. BMJ. 2005 Mar 26;330(7493):699. Epub 2005 Mar 9.

Papers with Title and Text in Languages Other Than English
Li YJ, He X, Liu LN, Lan YY, Wang AM, Wang YL. [Studies on chemical constituents in herb of Polygonum orientale]. Zhongguo Ahong Yao Za Zhi. 2005 Mar;30(6):444-6. Chinese.

Supplements or Special Editions
Pucca Junior GA, Lucena EHG, Cawahisa PT. Financing national policy on oral health in Brazil in the context of the Unified Health System. Braz Oral Res. 2010 Aug;24 Spec Iss 1:26-32.

Online Journals
Barata RB, Ribeiro MCSA, De Sordi M. Desigualdades sociais e homicídios na cidade de São Paulo, 1998. Rev Bras Epidemiol. 2008;11(1):3-13 [cited 2008 Feb 23]. Available from: http://www.scielosp.org/pdf/rbepid/v11n1/01.pdf.

Books
Stedman TL. Stedman's medical dictionary: a vocabulary of medicine and its allied sciences, with pronunciations and derivations. 20th ed. Baltimore: Williams & Wilkins; 1961. 259 p.

Books Online
Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Websites
Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000 [cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Instituto Brasileiro de Geografia e Estatística [homepage]. Brasília (DF): Instituto Brasileiro de Geografia e Estatística; 2010 [cited 2010 Nov 27]. Available from: http://www.ibge.gov.br/home/default.php.

World Health Organization [homepage]. Geneva: World Health Organization; 2011 [cited 2011 Jan 17]. Available from: http://www.who.int/en/

Anexo D

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

INSTRUÇÃO NORMATIVA N.º 014-CPPGCO, de 14 de março de 2013.

Dispõe sobre as Normas para Redação de Dissertações e Teses do Programa de Pós-Graduação em CIÊNCIA ODONTOLÓGICA, de acordo com a Resolução UNESP-24, DE 24/02/2012.

O Coordenador do Programa de Pós-Graduação em Ciência Odontológica desta Faculdade, considerando a decisão do Conselho do Programa, por ocasião de sua reunião, levada a efeito em 08/03/2013, baixa a seguinte instrução normativa:

Artigo 1º - A redação do trabalho de Dissertação ou Tese do aluno do Programa de Pós-Graduação em Ciência Odontológica poderá ser realizada pela forma tradicional ou em formato de artigo.
Artigo 2º - O trabalho em formato de artigo deverá conter os seguintes elementos em sua estrutura:
I – Trabalho resultando em somente um artigo
1. Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo em Português;
9. Título e Resumo em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Artigo na íntegra (redigido nas normas do periódico escolhido)
15. Anexos

II – Trabalho resultando em dois artigos ou mais
1.Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo Geral em Português;
9. Título e Resumo Geral em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Introdução Geral (com referências inseridas como notas de rodapé);
15. Artigos na íntegra (redigido nas normas do periódico escolhido)
16. Anexos

Parágrafo único – Os anexos deverão obrigatoriamente conter as normas dos periódicos nos quais os artigos foram redigidos. Além disso, toda a informação que vier a complementar o trabalho e que não constar no texto do artigo deverá ser colocada sob a forma de anexos. Estes poderão incluir, mas não se limitam a, imagens, fluxogramas, protocolos laboratoriais, tabelas, etc.
Artigo 3º - Esta instrução normativa entra em vigor na data de sua publicação.

Conselho do Programa, 14 de março de 2013.

Prof. Adj. ALBERTO CARLOS BOTAZZO DELBEM
Coordenador do Programa