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ANÁLISE COMPARATIVA DA AÇÃO ANTIMICROBIANA DOS CIMENTOS ENDODÔNTICOS ACROSEAL, SEALAPEX E AH PLUS PÓS PRESA EM BIOFILME DE *ENTEROCOCCUS FAECALIS*.

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

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Dados Curriculares

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Resumo

Rezende, GC. Análise comparativa da ação antimicrobiana dos cimentos endodônticos Acroseal, Sealapex e AH Plus pós presa em biofilme de *Enterococcus faecalis*. Araçatuba, 2015. 46p. Dissertação (Mestrado em Endodontia) – Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Resumo

Enterococcus faecalis é uma bactéria Gram-positiva que pode resistir ao tratamento endodôntico e manter a infecção presente no sistema de canais radiculares. Assim, o uso de cimentos endodônticos com atividade antimicrobiana pode ajudar a eliminar microrganismo residuais que sobreviveram após o tratamento endodôntico. Diante dessa realidade, o presente estudo teve por objetivo avaliar comparativamente a ação antimicrobiana dos cimentos endodônticos Acroseal, Sealapex e AH Plus em um modelo de biofilme *in vitro*. Para a realização do estudo foram confeccionados 144 espécimes de dentina bovina, sendo doze blocos para cada cimento em cada tempo experimental que permaneceram em placas contendo meio de cultura inoculado com *E. faecalis* (ATCC 51299) para permitir a formação de biofilme. Decorrido 14 dias, os espécimes foram transferidos para outra placa onde discos dos cimentos testados foram colocados sobre o biofilme formado. As placas de cultura foram deixadas em estufa a 37°C e 5% de CO_2 por 2, 7 e 14 dias. Espécimes sem aplicação dos cimentos foram utilizados como controle para cada período. Após cada tempo experimental, as amostras foram agitados em sonicador. As suspensões foram agitadas em vortex e foi realizada uma diluição seriada decimal em solução salina, sendo estas diluições plaqueadas em triplicata em meio de cultura m-Enterococcus ágar. As unidades formadoras de colônia foram contadas e os dados analisados estatisticamente usando os teste ANOVA, Shapiro-Wilk e Kruskal-Wallis one-way ($p<0,05$) para determinação do potencial antimicrobiano. **Resultados:** O Sealapex apresentou diferença estatística em todos os tempos experimentais quando comparado com os demais grupos. O AH Plus e o Acroseal mostraram atividade antimicroabina apenas no 14^{o} dia de experimento. **Conclusão**: Nenhum dos cimentos testados pós presa, foi capaz de eliminar o biofilme. O Sealapex mostrou maior atividade antimicrobiana em todos os períodos experimentais. Em todos os cimentos analisados a efetividade antimicrobiana foi maior com o decorrer do tempo.

Palavras chaves: Endodontia, Cimentos dentários, Biofilmes, *Enterococcus faecalis*.

Abstract

Rezende, GC. Comparative analysis of the antimicrobial action of endodontic sealer Acroseal, Sealapex and AH Plus setting against *Enterococcus faecalis* biofilm. Araçatuba, 2015. 46p. Dissertation (Master in Endodontics) – Dental School of Araçatuba, São Paulo State University "Júlio de Mesquita Filho".

Abstract

Enterococcus faecalis is a gram positive bacteria that can resist to the endodontic therapy and maintain the infection present in the root canal system. Therefore, the use of endodontic sealers with antimicrobial activity could help to eliminate residual microorganism that have survived after endodontic treatment. The present study aimed to compare the antimicrobial activity of the endodontic sealers Acroseal, Sealapex and AH Plus *in vitro* biofilm model. It were prepared 144 bovine dentin specimens, twelve blocks for each cement in each experimental time that remained in plates containing culture medium inoculated with *E. faecalis* (ATCC 51299) for biofilm formation. After 14 days, the samples were transferred to another plate with test sealers and were kept at 37^{o}C and 5% CO_2 for 2, 7 and 14 days. Specimens without sealers were used as a control for each period. After each experimental, the samples were agitated in sonicator. The suspensions were agitated in vortex and serially diluted in saline and triple plaqued in m-Enterococcus agar. Colony forming unit were counted and the data were statistically analyzed using ANOVA, Shapiro-Wilk and Kruskal-Wallis one-way test ($p<0.05$) for the antimicrobial potential determination. **Results:** Sealapex showed significant difference in all experimental times in comparison to all other groups. AH Plus and Acroseal showed antimicrobial activity just at 14^{th} experimental day. **Conclusion:** Neither of sealers setting tested was able to remove biofilm . The Sealapex showed higher antimicrobial activity in all experimental periods. All sealers examined the antimicrobial effectiveness was higher with over time.

Key words: Endodontics, Dental cements, Biofilms, *Enterococcus faecalis*.

Lista de Abreviações

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

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Artigo

Comparative analysis of the antimicrobial action of endodontic sealers Acroseal, Sealapex and AH Plus setting against *Enterococcus faecalis* biofilm.

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The manuscript is according to the guide lines for authors of Journal of Endodontic (Anexo B).

Abstract

Introduction: *Enterococcus faecalis* is a gram positive bacteria that can resist to the endodontic therapy and maintain the infection present in the root canal system. Therefore, the use of endodontic sealers with antimicrobial activity could help to eliminate residual microorganism that have survived after endodontic treatment . The present study aimed to compare the antimicrobial activity of the endodontic sealers Acroseal, Sealapex and AH Plus in vitro biofilm model. It were prepared 144 bovine dentin specimens, twelve blocks for each cement in each experimental time that remained in plates containing culture medium inoculated with *E. faecalis* (ATCC 51299) for biofilm formation. After 14 days, the samples were transferred to another plate with test sealers and were kept at 37ºC and 5% CO2 for 2, 7 and 14 days. Specimens without sealers were used as a control for each period. After each experimental, the samples were agitated in sonicator. The suspensions were agitated in vortex and serially diluted in saline and triple plaqued in m-Enterococcus agar. Colony forming unit were counted and the data were statistically analyzed using ANOVA, Shapiro-Wilk and Kruskal-Wallis one-way test ($p<0.05$) for the antimicrobial potential determination. Results: Sealapex showed significant difference in all experimental times in comparison to all other groups. AH Plus and Acroseal showed antimicrobial activity just at 14th experimental day. Conclusion: Neither of sealers setting tested was able to remove biofilm. The Sealapex showed higher antimicrobial activity in all experimental periods. All sealers examined the antimicrobial effectiveness was higher with over time.

Key Words: Endodontics, Dental cements, Biofilms, *Enterococcus faecalis*.

Introduction

Endodontic therapy aims to eliminate microorganisms from the root canal system throughout shaping, irrigation, and dressing. However, microorganism can not be completely eliminate (1).

Sundqvist et al. (2) related that 38% of failed root canal treatments were contaminated with *E. faecalis.* It is gram positive cocci, facultative anaerobes that overcome the challenges of survival within the root canal system by possessing the ability to survive very harsh environments including extreme alkaline pH (11,5) (3), to be small invades and lives within dentinal tubules (4), can to endure prolonged periods of nutrients (5). *E. faecalis* is also able to form a biofilm become more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms (6), and for these reasons is one of the most studied microorganisms in the area of endodontics.

The use of root canal filling materials with antibacterial activity collaborates for the elimination of remaining microorganisms (7). Several types of the endodontic sealers are commercially available, such as calcium hydroxide-based Acroseal and Sealapex and the epoxy resin-based AH Plus (8).

Sealapex and Acroseal are calcium hydroxide containing sealers and their dissociation in to calcium and hydroxyl ions become alkaline the environment and unfavorable for bacterial proliferation, including *E. faecalis* (1). The calcium ions can react with carbon dioxide and reduce the source of breathing to anaerobic bacteria (9).

AH Plus is a sealer with antimicrobial activity against *F. nucleatum* and *P. gingivalis* in the agar diffusion test (ADT) (10), and *E. faecalis* in the direct contact test (DCT) (11). It was also showed that Acroseal was effective against *E. faecalis* by formation of a zone of inhibition of microbial growth (12). However, according to manufacturer's information, its original formula was modified (where glycyrrhetic acid was replaced by TCD-diamide), and there may be changes in their antimicrobial properties. Therefore it is essential to establish whether the new formulation maintains the antimicrobial properties.

Some methodologies have been used to evaluate the antimicrobial action of endodontic filling materials, such as DCT (1, 7, 11), ADT (11, 12, 13, 14, 15, 16, 17), human or bovine teeth block model (18, 19, 20) and confocal laser (19). Most of these methodologies have used a planktonic microorganisms to test the sealers antimicrobial activity (11, 13, 14, 15, 16, 17, 21). However, the biofilm model is more appropriated once when organized into biofilme, microorganisms have greater resistance against materials activity antimicrobial (15). Therefore, the aim of the present study was to evaluate *in vitro* antibacterial activity of 3 root canal filling materials (Acroseal, AH Plus and Sealapex) by a direct contact test against *E. faecalis* biofilm.

Materials and methods

Sealer samples

The sealers used for the present study were Acroseal (Septodont), Sealapex (SybonEndo) and AH Plus (Dentsply). Sealer samples were made by manipulating and inserting the sealers to be tested into silicone sterile molds measuring 7 mm x1 mm (internal diameter x thickness) in a laminar flow chamber (Veco Bioseg 12 Ltda., Campinas,SP, Brazil). Specimens were kept at 37°C in controlled humidity for 56 hours. The sealers (Acroseal, Sealapex and AH Plus) were prepared according to the manufacturer recommendations.

Analysis of antibiofilm activity

Bovine incisors with completely formed roots were used as 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 of a diamond disc at low speed, under abundant irrigation. The resulting blocks were immersed in EDTA 17% for 3 minutes to remove the smear layer, after 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,SP, 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 agar (BHI, Difco Laboratories Inc., Detroit, MI, USA) and kept at 37°C for 24 hours. The colony was inoculated in to 5.0 mL sterile Brain Heart Infusion broth (BHI, Difco Laboratories Inc., Detroit, MI, USA) and kept at 37°C overnight, when the medium optical density was measured with a spectrophotometer (Eon Microplate Spectrophotometer, BioTek Instruments, USA) set at 550 nm wavelength. The optical density was adjusted in 0.06 (approximately 9 x 10^7 colony-forming units per mL (CFU mL^{-1})).

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 a 37°C 5% CO_2 (Incubator Ultra Safe, HF212-UV) at 37°C for 14 days (22). BHI medium of each specimen was completely changed every 48 h without adding new microorganisms.

Each endodontic sealer sample was removed from the mold and positioned over one of the dentine blocks containing biofilm. The dentine block/material sample were placed on new 24-well cell culture plates and kept at 37°C 5% CO_2 for 2, 7 and 14 days. Twelve root canal sealers discs were used per material and period of contact. In the control group, the biofilms were not placed in contact to any endodontic sealer discs. Pilot studies have shown the need to hydrate the dentine block/material samples with 20µL of saline every day to try simulating the root canal environment.

After the contact periods, the endodontic sealer discs were removed, and the dentine blocks containing the remaining biofilm including those belonging to the control group, were individually stored in test tubes containing 1 mL of sterile saline. The tubes were agitated with a sonicater (model S4000, Misonix, Inc., Fransingdale, NY, EUA) for 30 s at 40 W to disrupt the biofilm.

The obtained suspensions of *E. faecalis* were serial diluted and 10 µL aliquots of each suspension was inoculated onto Petri dishes containing M-Enterococcus agar medium (Acumedia, Neogen Corporation, Lansing, Michigan) and the dishes were incubated at 37°C 5% CO_2 for 48 h in triplicate.

The readings for each petri dish was performed on bacterial growth areas, where the generated dilutions between 3 and 30 colonies. The number of CFU mL^{-1} was calculated for each group and data were presented as the mean and standard deviation of the twelve specimens in each group.

Statistical Analysis

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

Results

Table 1 shows the mean CFU mL^{-1} after 2, 7 and 14 days of biofilms contact with sealers. Comparison between materials revealed that Sealapex reduced *E. faecalis* in 7 days and 14 days compared to 2 day ($p < 0.05$). AH Plus and Acroseal reduced the CFU counting just after 14 days when compared to the control group. Comparison between periods revealed that all sealers evaluated reduced *E. faecalis* CFU at the 14^{th} day in direct contact. *E. faecalis* in biofilm was not completely eliminated, regardless the sealer used or the period of time.

Discussion

In this study the hypothesis was that none of the material presented antimicrobial activity against biofilms of *E. faecalis*. This hypothesis was rejected because, all sealers tested showed antimicrobial activity against biofilm after 14 days of direct contact, where the Sealapex showed the best results. This antibacterial activity of sealers might help to eliminate residual microorganisms that have survived after endodontic treatment, increasing the chances of success (7). The aim of this study was the evaluation of the antibacterial activity of three endodontic sealers by a direct contact test in the biofilm against *E. faecalis* in a period of 2, 7 and 14 days. Although the antimicrobial activity of sealers has been used to evaluate the ADT, it is relatively insensitive, and the results are dependent upon diffusion and physical properties of the

tested materials (23). DCT was chosen in the present study due to be designed to overcome these limitations to assess the *in vitro* antibacterial activities of numerous endodontic sealers (24).

In the present study, Sealapex showed crescent antimicrobial activity along the time and it was significantly higher than Acroseal and AH Plus. Sealapex is a calcium hydroxide-based sealer that showed excellent antimicrobial activity in a period of 24h, 48h, 72h and 7 days by agar diffusion (13, 14, 15, 16, 23). Heyder et al. (11) evaluated the antimicrobial action of several sealers through DCT and shows that *E. faecalis* was influenced by Sealapex at 24h by DCT. In this study Sealapex showed high antimicrobial activity against *E. faecalis* on 7 and 14 days that was improved with time and presented the best result after 14 days of DCT with the biofilm. These results are according to Zhang et al. (7) that reported Sealapex possessed strongest antimicrobial effect against *E. faecalis* after 7 days in DCT with sealer. Similar results were found by Faria-junior et al. (18) with similar methodology this study, using DCT against *E. faecalis* biofilm and showed that Sealapex was associated with a higher reduction than the other materials on 7 days compared to the control group and with higher bacterial reduction with time. The ionization may be responsible for the antimicrobial activity of Sealapex that releases hydroxyl ions increasing the pH and leaving the environment unfavorable for microorganisms growth (25). Schäfer et al. (26) showed that Sealapex was significantly more soluble in water than AH Plus, which can to explain the antimicrobial action of Sealapex after 2 days.

Acroseal also is a calcium hydroxide-based sealer, but in the present study it did not show antimicrobial activity against *E. faecalis* with 2 and 7 days, but just after 14 days Pinheiro et al. (12) conducted an experiment by ADT with some endodontic sealers, the authors related Acroseal sealer were effective against *E. faecalis* at 48h. However, disagreed with the results of the present study, which can be explained by the different methodologies. The antimicrobial activity of hydroxide-based sealers may be caused by the release of hydroxyl ions, making the alkaline medium, however the Acroseal showed the least amount of calcium and hydroxyl ion release, due the relative insolubility of its compounds (9). The insolubility of its ingredients and low solubility (27) of Acroseal can explained the difference with Sealapex, although both were hydroxide-based sealers, and late antimicrobial activity, suggesting that the Acroseal need a longer time to release hydroxyl ion. There are few studies evaluating the antimicrobial activity of Acroseal, and further studies should be conducted in order to investigate its antibacterial activity.

In the present study it was not found antimicrobial activity for AH Plus after 2 and 7 days of DCT, these results agree with others studies (1, 28). Poggio et al. (17), used ADT with freshly mixed endodontic sealer (Endomethasone C, Argoseal, Bioseal Normal, Acroseal, AH Plus, Sicura Seal) after 24 and 48h, and showed low antibacterial effects for AH Plus in the tested periods. Wang et al. (19) evaluated the proportions of dead and live bacteria inside the dentinal tubules after exposure to root canal sealers were assessed by confocal laser scanning microscopy and related antimicrobial activity for AH Plus on 1 and 7 days, different to the results of the present study that did not find antimicrobial activity on 7 days. Such difference can be explained because in the

present study, the sealers was not freshly put in contact with the biofilm, the antimicrobial activity of the tested sealers depends on the time interval between mixing and testing (28). In the present study the sealers were put in contact to biofilm just 56 hours after manipulation while Wang et al. (19) and Poggio et al. (17) used the sealer freshly manipulate. Studies report that antimicrobial action of AH Plus is due to formaldehyde release over a short period of time during setting time (7, 25, 29). However, just on 14 days, AH Plus showed antimicrobial effectiveness against *E. faecalis* in the present study, disagreeing to Slutzy-Goldberg et al. (30) that did not find antimicrobial action after 14 days. The difference methodologies used can be explicated the variation of the results. The late activity antimicrobial for AH Plus can be explained because, recently study showed that this sealer present a low, discourages the occurrence of an alkaline pH and the release of calcium ions (31).

This study also showed that time is an important factor that influences the sealer antimicrobial action. All sealers showed some antimicrobial activity in direct contact compared to control group but just Sealapex showed significant antimicrobial action. After 14 days, Acroseal and AH Plu started its antimicrobial action but it was less effective than Sealapex.

Conclusion

Based on the results and within the limitations of the methodology, it could be concluded that E. faecalis in biofilm was not completely eliminated, regardless the sealer used or the period of time; AH Plus and Acroseal started its antimicrobial action just at 14th day; Sealapex showed higher antimicrobial activity against *E. faecalis* in all periods of time; and all sealers examined the antimicrobial effectiveness was higher with over time.

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

Days	Groups (n=12)			
	Acroseal	**AHplus**	**Sealapex**	**Controle**
2	$7.7x10^{6}$ ($\pm 7.4x10^{6}$)$^{A, a}$	$1.1x10^{7}$($\pm 1.0x10^{7}$)$^{A, a}$	$5.8x10^{5}$($\pm 5.0x10^{5}$)$^{B, a}$	$1.3x10^{7}$($\pm 1.3x10^{7}$)^{A,a}
7	$4.0x10^{6}$ ($\pm 3.5x10^{6}$)$^{A, a}$	$5.0x10^{6}$($\pm 3.7x10^{6}$)$^{A, a}$	$1.3x10^{4}$($\pm 1.1x10^{4}$)$^{B, b}$	$1.1x10^{7}$($\pm 1.1x10^{7}$)^{A,a}
14	$9.2x10^{5}$ ($\pm 9.6x10^{5}$)$^{A, b}$	$7.1x10^{5}$($\pm 7.9x10^{5}$)^{A,b}	$3.6x10^{1}$($\pm 8.4x10^{1}$)$^{B, c}$	$1.2x10^{7}$($\pm 1.6x10^{7}$)$^{C, a}$

*Different capital letters indicate statistical difference (P <0.05) among the groups.

*Different lower letters indicate statistical difference (P<0.05) among the periods of time.

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 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 estéril, colocar os cimentos e hidratar cada poço com 20µL de solução salina;

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

Dia 19, 24 e 31: Remover todo o meio com ponteira estéril;

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 33: Contar as placas (3-30 colônias):

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

Anexo C

Guide for Authors (Journal of Endodontics)

Introduction

The *Journal of Endodontics* is owned by the American Association of Endodontists. Submitted manuscripts must pertain to endodontics and may be original research (eg, clinical trails, basic science related to the biological aspects of endodontics, basic science related to endodontic techniques, case reports, or review articles related to the scientific or applied aspects of endodontics). Clinical studies using CONSORT methods (http://www.consort-statement.org/consort-statement/) or systematic reviews using meta-analyses are particularly encouraged. Authors of potential review articles are encouraged to first contact the Editor during their preliminary development via e-mail at *JEndodontics@UTHSCSA.edu*. Manuscripts submitted for publication must be submitted solely to *JOE*. They must not be submitted for consideration elsewhere or be published elsewhere.

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Before You Begin

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Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the randomization procedure. The CONSORT checklist and template flow diagram can be found on http://www.consort-statement.org.

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Preparation

General Points on Composition

Authors are strongly encouraged to analyze their final draft with both software (eg, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery because their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision or that have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the Journal of Endodontics:
a. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section "c," below), or sentences with little to no transition within a paragraph.

b. Keep to the point. The subject of the sentence should support the subject of the paragraph For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al, reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al, 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.

c. Sentences are stronger when written in the active voice, that is, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

d. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus, or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)
e. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary

instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.
f. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

g. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

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Abstract Headings

Introduction, Methods, Results, Conclusions

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Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Acknowledgements

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The authors deny any conflicts of interest related to this study.

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The Abstract concisely describes the purpose of the study in 250 or fewer words. It must be organized into sections: Introduction, Methods, Results, and Conclusions. The

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Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

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1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun. 2010;163:51–59.

Book:

2. Strunk W Jr, White EB. *The Elements of Style*, 4th ed. New York: Longman; 2000. Chapter in an edited book:

3. Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, eds. *Introduction to the Electronic Age.* New York: E-Publishing; 2009:281–304.
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Anexo D

unesp UNIVERSIDADE ESTADUAL PAULISTA
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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