Guilherme Hiroshi Yamanari

Influência do veículo na remoção da pasta tri antibiótica do canal radicular

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 Doutor em Endodontia.

Orientador: Prof. Titular João Eduardo Gomes Filho

Araçatuba - SP
2015

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

Catalogação na Publicação
Serviço Técnico de Biblioteca e Documentação - FOA / UNESP

Y21i

Yamanari, Guilherme Hiroshi
Influência do veículo na remoção da pasta tri antibiótica do do canal radicular / Yamanari Guilherme Hiroshi. – Araçatuba, 2015.
49 f. : il.; tab. + 1 CD-ROM.

Tese (Doutorado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba.
Orientador: Prof. João Eduardo Gomes Filho

1. Endodontia. 2. Regeneração. 3. Irrigação Terapêutica. 4. Antibioticoprofilaxia. I. T.

Black D24
CDD 617.67

Dados Curriculares

Guilherme Hiroshi Yamanari

Nascimento	04 de setembro de 1976 Araçatuba/SP
Filiação	Massako Tanaka Yamanari Hiroshi Yamanari
1995-1998	Curso de Graduação em Odontologia – Universidade de Marília/SP UNIMAR
2001-2003	Curso de Especialização em Endodontia –Faculdade de Odontologia de Araçatuba – Universidade Estadual Paulista ~~–~~Júlio de Mesquita Filho" – UNESP
2001-2002	Curso de Pós-Graduação em Endodontia, nível de Mestrado – Universidade de Marília/SP UNIMAR
2012-2015	Curso de Pós-Graduação em Endodontia, nível de Doutorado – Faculdade de Odontologia de Araçatuba – Universidade Estadual Paulista ~~–~~Júlio de Mesquita Filho" – UNESP

Dedicatória

Dedico este trabalho,

À família Hiroshi Yamanari,

Meu pai, Hiroshi, meu porto seguro!

Minha mãe, Massako, minha inspiração !

Minha esposa, Letícia, meu entusiasmo !

Minha Irmã, Ariane, meu foco!

Arthur, Rodrigo e Heitor, meus filhos e sobrinho, minha vida !

Grato por sempre acreditarem,

Amo vocês!

Agradecimentos

À Deus, pelo dom da vida e por ter me dado tantas oportunidades, como esta.

Aos meus sogros, Gerson e Sônia, pelo incentivo.

Ao meu cunhado, Rodrigo, pela amizade e apoio.

Aos meus padrinhos Kaneo e Yaeko, pela torcida e carinho.

Aos tios e primos que sempre me apoiaram em momentos de dificuldade.

Aos professores do departamento de Endodontia da FOA, Dr. Luciano Tavares Angelo Cintra e Dr Elói Dezan Jr, grato pela confiança e pelo acolhimento.

Agradeço nas pessoas, do Dr. Arnaldo Sant'Anna Jr, Dr. Marco Antônio Hungaro Duarte, Dra. Sâmira Âmbar Lins, Sônia Âmbar Amaral e Paulo Valério Lins, a todos os meus amigos, pela solidariedade e pelo crescimento.

À Dra. Carolina Simonetti Lodi, e na sua pessoa a todos os professores, alunos e funcionários do Curso de Odontologia da FUNEC.

À Gabriely Cristinni Rezende e Marcelo Tadahiro Wayama, e em seus nomes, todos os colegas do curso de pós graduação da Faculdade de Odontologia de Araçatuba.

Agradecimento Especial

Ao meu amigo e orientador,
Prof. Titular Dr. João Eduardo Gomes Filho,
pela serenidade em transmitir seus conhecimentos e pelos anos de dedicação e incentivo.

Muito Obrigado!

Resumo

Yamanari, GH. Influência do veículo na remoção da pasta tri antibiótica do canal radicular. Araçatuba, 2015.49p. Tese (Doutorado em Endodontia) – Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista –Júlio de Mesquita Filho".

Resumo

OBJETIVO: Este estudo avaliou o efeito do veículo na eficácia da irrigação com agulha associada ou não com irrigação ultrassônica passiva (IUP) na remoção da pasta tri antibiótica do canal radicular.

MÉTODOS: O canal radicular de 60 dentes uniradiculares foi preparado com instrumento rotatório ProTaper até a lima F4. Vinte canais radiculares foram obturados com pasta triantibiótica (PTA), preparados com macrogol mais propilenoglicol, água destilada ou propilenoglicol. Após 4 semanas de armazenamento em estufa, foi realizada irrigação com agulha utilizando hipoclorito de sódio a 2,5%, associada ou não com a IUP para a remoção da PTA. Seis grupos de 10 espécimes foram tratados de acordo com o protocolo de veículos e de irrigação. As raízes foram divididas longitudinalmente e a quantidade de pasta antibiótica restantes foi avaliada através de estereomicroscópico com aumento de 20 vezes e por MEV utilizando um sistema de pontuação 4-grades. Os dados foram avaliados estatisticamente através dos testes Kruskal Wallis e Dunn`s com um nível de confiança de 95% ($p = 0,05$).

RESULTADOS: O uso da IUP não melhorou a remoção da PTA ($p > 0,05$). Não houve diferença estatisticamente significante entre os terços coronal, médio e apical($p > 0,05$). Nos terços médio e apical, a PTA com propilenoglicol como veículo apresentou mais resíduos do que a PTA preparada com água destilada ($p < 0,05$). Água destilada ou macrogol mais propilenoglicol foram semelhantes independentemente do terço do canal radicular considerado.

CONCLUSÕES: Não foi possível remover completamente a PTA dos canais radiculares mesmo com a utilização da IUP. Água destilada ou macrogol mais propilenoglicol são melhores como veículo para favorecer a remoção de qualquer resíduo da PTA.

Palavras chaves: Irrigação Terapêutica, Antibioticoprofilaxia, Regenereção, Endodontia.

Abstract

Yamanari, GH. Vehicle influence on the removal of triple antibiotic paste from the root canal. Araçatuba, 2015.49p.Thesis (PhD in Endodontics) – Dental School of Araçatuba, São Paulo State University –Júlio de Mesquita Filho".

Abstract

AIM: This study evaluated the interference of the triple antibiotic paste vehicle on its removal from the root canal using needle irrigation with or without passive ultrasonic irrigation (PUI).

METHODS: Sixty root canals were prepared up to F4 ProTaper and were filled with triple antibiotic pastes (TAP) prepared with macrogol plus propylene glycol, distilled water, or propylene glycol. After 4 weeks of stove storage, needle irrigation with 2.5% sodium hypochlorite with or without PUI was used for TAP removal. The roots were longitudinally split and the amount of remaining antibiotic pastes and cleaning of the dentinal tubule were evaluated under a stereomicroscope and SEM. The data were statistically evaluated using the Kruskal Wallis and Dunn`s tests ($p = .05$).

RESULTS: In the middle and apical thirds, TAP with propylene glycol remained more than that prepared with distilled water ($p < .05$). Distilled water or macrogol plus propylene glycol were similar independently of the root canal third. The use of PUI did not improve the removal of the TAP ($p > .05$). There were no statistically significant differences between the coronal, medium or apical thirds ($p > .05$).

CONCLUSIONS: None vehicle allowed the complete paste removal and cleaning of the dentinal tubule from the root canal and despite the use of PUI. Distilled water or macrogol plus propylene glycol as vehicle favored smaller remnant of TAP in the apical and middle thirds.

Keywords: Therapeutic Irrigation, Antibiotic Prophylaxis, Regeneration, Endodontics.

Lista de Figuras

Lista de Figuras

Lista de Tabelas

Lista de Tabelas

Sumário

Sumário

Artigo

Vehicle influence on the removal of triple antibiotic paste from the root canal.

Gomes-Filho JE[1], Yamanari GH[2], Cassucci PP[2], Wayama MT[1], Lodi CS[3], Cintra LTA[1], Guimarães BM[4], Duarte MAH[4].

[1]Department of Endodontics, Araçatuba Dental School, Universidade Estadual Paulista (UNESP), R. José Bonifácio, 1193, 16015-050, Araçatuba/São Paulo, Brazil.
[2]Department of Endodontics, Dentistry School in Santa Fé Foundation, Av. Mangará, 477, 15775-000, Santa Fé do Sul/São Paulo, Brazil.
[3]Department of Pediatric Dentistry, Dentistry School in Santa Fé Foundation, Av. Mangará, 477, 15775-000, Santa Fé do Sul/São Paulo, Brazil.
[4]Department of Endodontics, Bauru School of Dentistry, Univ. São Paulo, Al. Octávio Pinheiro Brisola, 9-75, 17012-901, Bauru/São Paulo, Brazil.

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

Corresponding author:

Dr. João Eduardo Gomes-Filho
Endodontics,
Araçatuba School of Dentistry, Univ. Estadual Paulista,
R. José Bonifácio, 1193
Araçatuba- São Paulo
Brazil
Tel: (0055) 18 36363252
Fax: (0055) 18 36363279
e-mail:joao@foa.unesp.br

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

Abstract

AIM: This study evaluated the interference of the triple antibiotic paste vehicle on its removal from the root canal using needle irrigation with or without passive ultrasonic irrigation (PUI).

METHODS: Sixty root canals were prepared up to F4ProTaperand were filled with triple antibiotic pastes (TAP) prepared with macrogol plus propylene glycol, distilled water, or propylene glycol. After 4 weeks of stove storage, needle irrigation with 2.5% sodium hypochlorite with or without PUI was used for TAP removal. The roots were longitudinally split and the amount of remaining antibiotic pastes and cleaning of the dentinal tubule were evaluated under a stereomicroscope and SEM. The data were statistically evaluated using the Kruskal Wallis and Dunn`s tests ($p = .05$).

RESULTS: In the middle and apical thirds, TAP with propylene glycol remained more than that prepared with distilled water ($p < .05$). Distilled water or macrogol plus propylene glycol were similar independently of the root canal third. The use of PUI did not improve the removal of the TAP ($p > .05$). There were no statistically significant differences between the coronal, medium or apical thirds ($p > .05$).

CONCLUSIONS: None vehicle allowed the complete paste removal and cleaning of the dentinal tubule from the root canal and despite the use of PUI. Distilled water or macrogol plus propylene glycol as vehicle favored smaller remnant of TAP in the apical and middle thirds.

Keywords: Triple antibiotic paste, irrigation, pulp revascularization, PUI

Introduction

Regenerative endodontic treatment is a biologically based procedure aimed reestablishing a functional pulp-dentin complex. The key components include disinfection of the root canal and introduction of stem cells, growth factors, and scaffolds (1) using treatment methods that impose minimal toxicity to stem cells (2).

Antibiotic pastes have been used as dressing material during revascularization treatment (3, 4). Triple antibiotic paste (TAP) has been found to have antimicrobial properties and be biocompatible (5–8). It consists of ciprofloxacin, metronidazole, and minocycline and was developed by Hoshino et al (6).

However, it was demonstrated that TAP had negative effects more than on esthetic (9,10)but on apical papilla human stem cells (SCAP)(11, 12). It has been demonstrated that TAP at the paste-like concentrations typically used in regenerative endodontic procedures are lethal in direct and indirect contact with SCAP when prepared with distilled water as vehicle(11, 12). Its indirect effect could trigger the complete destruction of SCAP placed in a scaffold within the root canal lumen after washing(12). It was hypothesized that the negative effect maybe due to the residual paste remained on the dentin despite extensive efforts in washing out with irrigants(12).

The standard vehicle to prepare TAP to be used in revascularization is macrogol plus propylene glycol (6), as reported in several studies(4, 8, 13, 14, 15). However, the use of saline (7, 16), distilled water (3, 9, 11, 12), and propylene glycol (17) have also been reported. It is possible that a paste prepared with a vehicle with adequate physical-chemical properties combined to an efficient removal protocol can achieve the complete TAP removal from the root canal helping the stem cell survival leading to a satisfactory pulp revascularization or regeneration.

There are some standard irrigation protocols to remove medicaments from the root canal system. Sonic irrigation with EndoActivator (EA) has been reported to effectively clean debris from the canals and remove the smear layer even when used in curved canal systems (18, 19). Passive ultrasonic irrigation (PUI) uses ultrasonic activation of irrigants for the efficient removal of debris and microorganisms (20).Sodium hypochlorite (NaOCl), EDTA, and sterile saline solution have been used for the removal of antibiotic pastes(1, 21–23); however, their failure to completely

remove the TAP from the root canal was demonstrated, even when PUI was used (24, 25).

There is no study evaluating the influence of different vehicles on the removal of TAP from root canals. Thus, the present study evaluated the effect of the vehicle on the efficacy of needle irrigation with or without passive ultrasonic irrigation (PUI) on the removal of triple antibiotic paste from the root canal. The null hypothesis was that the vehicle did not interfere on TAP removal, independently of the irrigation method or root canal third.

Material and Methods

Tooth Collection and Preparation

The study included 60 single-rooted, single and oval shape root canal, non carious, human teeth with similar sizes and completed apices (Ethical Protocol # 613.331). Soft tissues and calculus were mechanically removed from the root surfaces with a periodontal scaler. Single-root canals were confirmed with radiographs, and the teeth were then stored in distilled water until use. Teeth were decoronated with a diamond bur under water coolant to obtain a standardized root length of 14 mm. The root canals were shaped with ProTaper rotary files (DentsplyMaillefer, Ballaigues, Switzerland) up to an F4 (D0 = .40 mm) and 1mm shorter than the root length. During the preparation, the root canals were irrigated with 2 mL of 2.5% NaOCl solution after each instrument. A final irrigation was applied using 5 mL of 17% EDTA for 30 seconds and 5 mL of 2.5% NaOCl for 60 seconds. The root canals were then dried with paper points (DentsplyMaillefer).

Application of Antibiotic Paste

Triple antibiotic paste was prepared using macrogol ointment plus propylene glycol (TM), distilled water (TW) or propylene glycol (TP) as a vehicle.

TM was prepared by mixing equal portions of USP-grade antibiotic powders metronidazole, ciprofloxacin, and minocycline (Apoticário, Araçatuba, SP, Brazil) with a vehicle consisting of equal parts of macrogol ointment and propylene glycol (powder/liquid ratio of 5:1 for a total of 1 g/ml triple antibiotic paste)(8).

TW was prepared by mixing equal portions of USP-grade antibiotic powders

metronidazole, ciprofloxacin, and minocycline (Apoticário, Araçatuba, SP, Brazil) with distilled water to a creamy consistence (9, 11, 12).

TP was prepared by mixing equal portions of USP-grade antibiotic powders metronidazole, ciprofloxacin, and minocycline (Apoticário, Araçatuba, SP, Brazil) with propylene glycol in a proportion of 7:4 powder/propylene glycol to obtain a paste-like consistency (17).

The root canals were filled with TAP using a lentulo instrument (DentsplyMaillefer). Access to the root canals was temporarily sealed with a cotton pellet and temporary restorative material (Coltosol, VigodentColtene SA Indústria e Comércio, Rio de Janeiro, RJ, Brazil), and the specimens were then kept at 37^{o}C with 100% humidity for 4 weeks. The specimens were divided randomly into 6 groups (n = 10) and irrigated as follows:

1. Needle irrigation-TM: 6 mL of 2.5% NaOCl via a size 27-G blunt-tip needle (Ultradent, South Jordan, UT). The needle was inserted into the root canal within 2 mm of the working length without binding (TMN group);
2. Needle irrigation–TW: TAP was removed from the root canals using the needle irrigation as stated previously (TWN group);
3. Needle irrigation–TP: TAP was removed from the root canals using the needle irrigation as stated previously (TPN group);
4. Needle plus PUI-TM: 6 mL of 2.5% NaOCl via a size 27-G blunt-tip needle (Ultradent, South Jordan, UT) was used for 60 seconds plus PUI with ultrasonic tip #20 (Irrisonic, Santa Rosa de Viterbo, São Paulo, Brazil) coupled to the file-holding adapter of the handpiece of a conventional dental ultrasonic device (ENAC Plus, Adiel LTDA, São Paulo, SP, Brazil) (TMP group);
5. Needle plus PUI-TW: TAP was removed from the root canals using the needle irrigation plus PUI as stated previously (TWP group);
6. Needle plus PUI-TP: TAP was removed from the root canals using the needle irrigation plus PUI as stated previously (TPP group).

After the 28-day incubation period, the coronal seal was removed, and the canals were irrigated according to the protocols. For the PUI group, the ultrasonic tip was used to oscillate inside the root canals (1 mm shorter than the working length) at 35kH for

30s. The total irrigation time was 2 minutes, divided into 3 sequences of 40 seconds. The 3 sequences were subdivided into 20-second ultrasonic activation and 20-second refreshment (26).

Removal analysis under stereomicroscope

The root canals were dried with paper points, and the roots were disassembled to evaluate the removal of the antibiotic pastes. The teeth were split along their long axis in a buccolingual direction using a hammer and chisel to observe the root canal halves.

Digital images at 20x magnification were obtained using a stereomicroscope (Olympus BX43; Olympus Co, Tokyo,Japan) attached to a digital camera (Olympus SC100;Olympus Soft Imaging Solution GmbH, Munster, Germany) and were transferred to the computer. The digital images were coded to avoid identifying the specimens. Two calibrated observers were blind to the technique used to remove the antibiotic pastes. Reference photographs were selected for each score. Calibration of the observers was then performed on the photographs of 50 root halves. After scoring, the results were also discussed using reference photographs. Finally, the observers performed the main evaluation. The amount of antibiotic pastes remaining in the root canals was scored using the following scoring system adapted to that described by van der Sluiset al(27): (0) root canal wall was completely clean, (1) dispersed particles (2) concentrated antibiotic paste was present on less than half of the wall area, (3) concentrated antibiotic paste covered more than half of the wall area, and (4) concentrated antibiotic paste completely covered the wall area.

Removal analysis under SEM

Subsequently for stereomicroscope analysis, the halves were randomly chosen for SEM preparation and analysis. The specimens were dehydrated in ascending ethanol concentrations and mounted on aluminum stubs. Next, they were coated with a film of gold palladium and examined in a scanning electron microscope (Aspex express, FEI, Eindhoven, Netherlands) operated at 10 kV. The cervical, medium and apical thirds of the roots were used to evaluate the removal of the paste after the irrigation protocols magnified by 1000x. The electron micrographs were obtained from the cervical (3 mm from the cervical limit) medium (7mm from the cervical limit) and apical thirds (3 mm from the apical constriction) of each root canal. They were then transferred and recorded on a CD and evaluated by two trained examiners blind to the irrigation

protocols and materials. Thus, each micrograph was scored using a semi quantitative analysis with a four step scale (28) as follows: (1) all dentinal tubules visible; (2) more than 50% of dentinal tubules visible; (3) less than 50% of dentinal tubules visible; and (4) no dentinal tubules visible.

Statistical analysis

The differences in the scores of antibiotic pastes among the different groups were analyzed with the Kruskal-Wallis, Dunn's and Mann-Whitney U tests. All testing was performed at the 95% confidence level (P = .05). All statistical analyses were performed using the Sigma Plot software.

Results

The concordance between the observers in the analysis of the images was great (kappa value=0.908), and the difference between the matched scores never exceeded 1 unit. Intra individual reproducibility was 99%. No method used was effective for complete TAP removal from the root canals.

Considering the results from the analysis under stereomicroscope, the Kruskal-Wallis test did not reveal significant differences between the groups for all thirds (cervical, middle and apical) ($p>0.05$). The Mann-Whitney U test also did not reveal significant differences between the 2 methods used for the removal of the TAP, independently of the third ($P >0.05$).On the other hand, the Kruskal-Wallis test showed significant differences regarding the vehicle in the middle and apical thirds. The Dunn`s test evidenced that in the middle third, the TP remained more than the TM ($p<0.05$) but not more than the TW ($p>0.05$). In the apical third, the TP remained more than the TM and TW, independently of the use of PUI ($p<0.05$). However the analysis under SEM did not show the same differences once most of the specimens showed smear layer covering the dentinal tubules ($p>0.05$).

The figure 1 presents SEM images of the apical third of the representative specimens of each group studied.

Discussion

The null hypothesis was partially rejected once TAP was not completely removed from the canal but the vehicle interfered on its removal, principally in the

apical and middle thirds.

The remaining effects of TAP on stem cell biology and the clinical outcome in regenerative endodontic procedures requires investigation since their negative effects on apical papilla human stem cells was already shown(11, 12).As a result, strategies or TAP compositions aiming to achieve its complete removal needs to be investigated.

Optical microscopy and SEM have been used to evaluate root canal cleaning(20, 24, 28, 29). The advantage of using SEM over optical microscopy is that SEM enables researchers to view both debris and the smear layer (29). However, in the present study, most of specimens showed smear layer and there was no difference between the groups, differently from the stereomicroscopic analysis that evaluated the remaining in a more wide view considering the debris and showed some differences specially regarding the different vehicles.

Positive pressure and PUI were chosen because of the very high removal rates detected by both techniques, resulting in approximately 90% and 95% removal, respectively (25). Positive pressure is the most widely used irrigation technique, and ultrasonic activation has been shown to be more effective for removing dressings(25). Passive ultrasonic irrigation (PUI) activates an endodontic file with an ultrasonic device inside the root canal to mechanically agitate the irrigant without contacting the root canal wall (20). During PUI, the energy of a freely oscillating file is transmitted to the irrigant within the root canal, which results in acoustic streaming (30). PUI is characterized by the non cutting action of the ultrasonic file during irrigation to avoid changes in the root canal anatomy (20). In the current study, it was shown that the TAP was not adequately removed from the root canals despite the use of different vehicles or irrigation techniques. Previous studies also reported that the TAP was not completely removed even when PUI was applied (24, 25, 31). The high diffusion and affinity of TAP for dentin may explain why this drug combination is difficult to remove and the resulting minocycline-mediated staining (25).

One important finding of the present study was the influence of a vehicle on the removal of the TAP. Propylene glycol as a vehicle for TAP was found to be difficult to remove, while distilled water and macrogol plus propylene glycol were better and similar to each other,especially in the apical third. Propylene glycol (1,2-propanediol), a dihydric alcohol (CH3CH(OH)CH2OH)is a vehicle used as a pharmaceutical solvent for preparations in medicine(32), including as a vehicle for calcium hydroxide in Endodontics(33).The difficulty of the TAP prepared with propylene glycol for removal

may be due to the ability of the propylene glycol to effectively penetrate the dentin faster than distilled water and its hygroscopic properties that allow absorption of water, resulting in a sustained release of intracanal medicaments for prolonged periods (31, 34).

Interestingly, when propylene glycol was mixed with polyethylene glycol (PEG) as a TAP vehicle, its removal was facilitated. PEG is also called macrogol (HO–(CH2–CH2–O)n–H) which is used as a base for washable ointments, suppository bases (mainly mixtures of various PEGs), vehicles for parenteral products and tablets having different uses, binders, plasticizers in coating systems and also as a lubricant (35). It is a product based on polycondensation of ethylene oxide and water becoming a large molecular size compound and has the ability to attract a water shell around the polymer because each ethylene glycol subunit attracts two or three water molecules facilitating the solubility (36). Such solubility may explain the improvement of the removal of the TAP prepared with propylene glycol plus macrogol.

Distilled water is one of the most common vehicles used in delivering medicaments into the root canal. It is an aqueous substance having a high viscosity and surface tension that causes a high degree of solubility when the paste that it forms with the medicament comes in direct contact with tissue and tissue fluids, resulting in its rapid solubilization and resorption by tissue macrophages (33, 34). Although its surface tension does not help penetration into the dentinal tubules (33), it is possible that the dehydration of the paste prepared with distilled water could have influenced its removal differently from the other vehicles that have better ointment properties(33).

Other vehicles and irrigation protocols need to be studied to improve the removal of the TAP from the root canal. It was possible to conclude that none TAP vehicle allowed its completely removed from root canals independently of irrigation protocol. Moreover, distilled water or macrogol plus propylene glycol as vehicle favored TAP removal.

References

1. Murray PE, Garcia-Godoy F, Hargreaves KM (2007). Regenerative endodontics: a review of current status and a call for action. J Endod33:377–90.

2. Diogenes A, Henry MA, Teixeira FB et al (2013)An update on clinical regenerative endodontics. Endod Top28:2–23.

3. Bezgin T, Yilmaz AD, Celik BN et al (2014). Concentrated platelet-rich plasma used

in root canal revascularization: 2 case reports. IntEndod J47:41–9.
4. Er K, Kustarci A, Ozan U et al (2007) Nonsurgical endodontic treatment of dens invaginatus in a mandibular premolar with large periradicular lesion: a case report. J Endod33:322–4.
5. Sato I, Ando-Kurihara N, Kota K et al (1996)Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. IntEndod J29:118–24.
6. Hoshino E, Kurihara-Ando N, Sato I et al (1996)In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. IntEndod J29:125–30.
7. Thibodeau B, Teixeira F, Yamauchi M et al (2007)Pulp revascularization of immature dog teeth with apical periodontitis. J Endod33:680–9.
8. Gomes-Filho JE, Duarte PC, de Oliveira CB et al (2012)Tissue reaction to a tri antibiotic paste used for endodontic tissue self-regeneration of non vital immature permanent teeth. J Endod38:91–4.
9. Kim JH, Kim Y, Shin SJ et al (2010) Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod36:1086–91.
10. Akcay M, Arslan H, Yasa B et al (2014) Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. J Endod 40:845–8.
11. Ruparel NB, Teixeira FB, Ferraz CC et al (2012)Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod38:1372–5.
12. Althumairy RI, Teixeira FB, Diogenes A (2014) Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. J Endod40:521-5.
13. Banchs F, Trope M (2004) Revascularization of immature teeth with apical periodontitis: new treatment protocol? J Endod30:196-200.
14. Ding RY, Cheung GS, Chen J et al (2009). Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J Endod35:745-9.
15. Jung I, Lee S, Hargreaves KM (2008) Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J Endod34:876-87.
16. Nosrat A, Homayounfar N, Oloomi K (2012) Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J Endod38:1428-34.
17. Ordinola-Zapata R, Bramante CM, Minotti PG et al (2013)Antimicrobial activity of

triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on an intraoral-infected dentin biofilm model. J Endod 39:115-8.
18. Rodig T, Dollmann S, Konietschke F et al (2010) Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod36:1983–7.
19. Kanter V, Weldon E, Nair U et al (2011). A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. Oral Surg Oral Med Oral Pathol Oral RadiolEndod112:809–13.
20. van der Sluis LW, Versluis M, Wu MK, et al (2007) Passive ultrasonic irrigation of the root canal: a review of the literature. IntEndod J40:415–26.
21. Zhu W, Zhu X, Huang GT et al (2013) Regeneration of dental pulp tissue in immature teeth with apical periodontitis using platelet-rich plasma and dental pulp cells. IntEndod J46:962–70.
22. Tawfik H, Abu-Seida AM, HashemAA et al (2013) Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. IntEndod J46:910–22.
23. Utneja S, Garg G, Arora S et al (2012) Nonsurgical endodontic retreatment of advanced inflammatory external root resorption using mineral trioxide aggregate obturation. Case Rep Dent 624–792.
24. Arslan H, Capar ID, Saygili G et al (2014) Efficacy of various irrigation protocols on the removal of triple antibiotic paste. IntEndod J47:594-9
25. Berkhoff JA, Chen PB, Teixeira FB et al (2014) Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod40:1172-7.
26. van der Sluis LW, Vogels MP, Verhaagen B et al (2010) Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod36:737-40.
27. van der Sluis LW, Wu MK, Wesselink PR (2007). The evaluation of removal of calcium hydroxide paste from an artificial standardized groove in the apical root canal using different irrigation methodologies. IntEndod J40:52–7.
28. Chopra S, Murray PE, Namerow KN (2008) A scanning electron microscopic evaluation of the effectiveness of the F-file versus ultrasonic activation of a K-file to remove smear layer. J Endod 34:1243–5.
29. Ribeiro EM, Silva-Sousa YT, Souza-Gabriel AE et al (2012) Debris and smear removal in flattened root canals after use of different irrigant agitation protocols.

Microsc Res Tech75:781–90.
30. Roy RA, Ahmad M, Crum LA (1994). Physical mechanisms governing the hydrodynamic response of an oscillating ultrasonic file. IntEndod J27:197–207.
31. Arslan H, Akcay M, Capar ID et al (2014) Efficacy of Needle Irrigation, EndoActivator, and Photon-initiated Photoacoustic Streaming Technique on Removal of Double and Triple Antibiotic Pastes. J Endod40:1439–42.
32.Lessmann H, Schnuch A, Geier J et al (2005) Skin-sensitizing and irritant properties of propylene glycol. Contact Dermatitis53:247-59.
33. Cruz EV, Kota K, Huque J et al (2002)Penetration of Propylene glycol into dentine. IntEndod J34:330-6.
34. Fava LRG, Saunders WP (1999) Calcium hydroxide pastes: classification and clinical indications. IntEndod J32:257–82.
35. Kibbe AH(2000) Handbook of Pharmaceutical Excipients. ThePharmaceutical Press,London.
36. Harris, JM,Chess, RB (2003). Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov 2:214–21.

Figure 1. SEM images of apical third after irrigant protocols: a. TWP; b. TWN c. TMP d. TMN e. TPP f. TPN

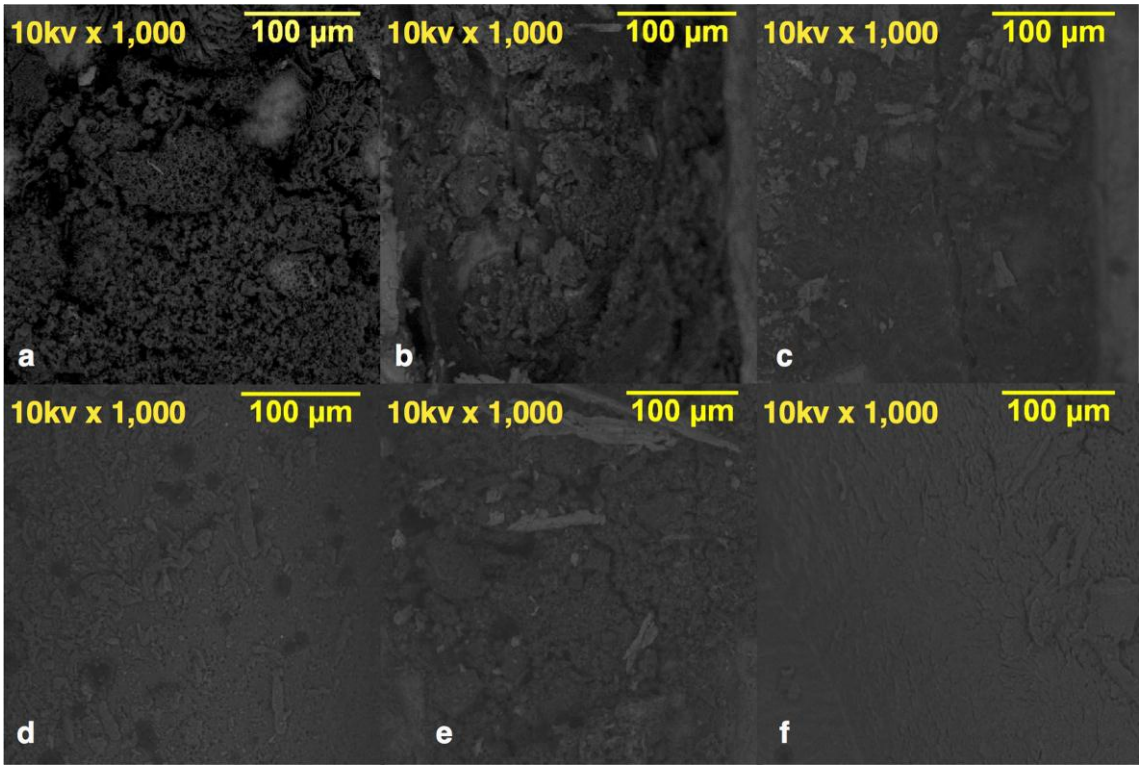

Table 1. Medians, Standard Error Means, and 25% to 75% Quartiles of the Groups considering the thirds

	Stereomicroscopicanalysis			SEM analysis		
	Median	Standard error	25%-75%	Median	Standard error	25%-75%
Apical						
TWP[a1]	2	0.339	1.75-3.25	4	0.300	2.00-4.00
TWN[a1]	2	0.388	1.00-3.25	3.5	0.221	3.00-4.00
TMP[a1]	2	0.276	1.00-2.00	3	0.249	2.00-3.25
TMN[a1]	2	0.276	1.00-3.00	2.5	0.290	2.00-4.00
TPP[a1]	3	0.442	2.50-4.25	3	0.276	2.00-4.00
TPN[a1]	3	0.300	2.75-4.00	4	0.266	2.75-4.00
Middle						
TWP[a1]	3	1.033	2.00-4.00	2	0.307	2.00-3.25
TWN[a1]	2	0.971	2.00-3.25	3	0.249	2.75-4.00
TMP[a1]	2	0.788	2.00-2.25	2	0.266	2.00-3.25
TMN[a1]	2	1.160	2.00-4.00	2	0.223	2.00-3.00
TPP[a1]	4	1.033	2.00-4.00	3.5	0.260	2.75-4.00
TPN[a1]	4	1.337	2.00-4.00	3	0.276	2.00-4.00
Coronal						
TWP[a1]	3	1.054	2.00-4.00	2	0.276	1.75-2.25
TWN[a1]	3	0.942	2.00-4.00	2.5	0.166	2.00-3.00
TMP[a1]	2	0.707	2.00-3.00	2	0.163	2.00-3.00
TMN[a1]	2	0.966	2.00-4.00	2	0.266	2.00-2.50
TPP[a1]	2	0.674	2.00-2.25	2	0.223	2.00-3.00
TPN[a1]	3	1.135	2.00-4.25	3	0.233	2.00-3.25

* Different letters and numbers mean statistical difference into thirds (leters for Stereomicroscopic and numbers for SEM analysis)

Table 2. Medians, Standard Error Means, and 25% to 75% Quartiles of the thirds considering the removal method

	Stereomicroscopicanalysis			SEM analysis		
	Median	Standard error	25%-75%	Median	Standard error	25%-75%
Apical						
PUI[a1]	2	0.223	1.75-3.25	3	0.158	2.00-4.00
Needle[a1]	3	0.207	1.75-3.00	3	0.154	2.00-4.00
Middle						
PUI[a1]	2	0.185	2.00-4.00	3	0.168	2.00-4.00
Needle[a1]	2.5	0.227	2.00-4.00	3	0.151	2.00-4.00
Coronal						
PUI[a1]	2	0.156	2.00-3.25	2	0.129	2.00-3.00
Needle[a1]	3	0.185	2.00-4.00	2	0.132	2.00-4.00

* Different letters and numbers mean statistical difference into thirds (leters for Stereomicroscopic and numbers for SEM analysis)

Table 3. Medians, Standard Error Means, and 25% to 75% Quartiles of the thirds considering the paste vehicle

	Stereomicroscopicanalysis			SEM analysis		
	Median	Standard error	25%-75%	Median	Standard error	25%-75%
Apical						
Water[a1]	2	0.252	1.00-3.00	4	0.181	3.00-4.00
Macrogol+ propylene glycol[a1]	2	0.191	1.00-2.75	3	0.186	2.00-3.75
Propylene glycol[b1]	3	0.260	3.00-4.00	3.5	0.190	2.25-4.00
Middle						
Water[ab1]	2.5	0.220	2.00-3.75	3	0.208	2.00-4.00
Macrogol+ propylene glycol[a1]	2	0.223	2.00-3.75	2	0.169	2.00-3.00
Propylene glycol[b1]	4	0.266	2.00-4.00	3	0.186	2.25-4.00
Coronal						
Water[a1]	3	0.217	2.00-4.00	2	0.163	2.00-3.00
Macrogol+ propylene glycol[a1]	2	0.184	2.00-3.00	2	0.152	2.00-3.00
Propylene glycol[a1]	2	0.228	2.00-3.00	3	0.163	2.00-3.00

* Different letters and numbers mean statistical difference into thirds (leters for Stereomicroscopic and numbers for SEM analysis)

Anexos

Anexo A

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.

Disclaimer

The statements, opinions, and advertisements in the *Journal of Endodontics* are solely those of the individual authors, contributors, editors, or advertisers, as indicated. Those statements, opinions, and advertisements do not affect any endorsement by the American Association of Endodontists or its agents, authors, contributors, editors, or advertisers, or the publisher. Unless otherwise specified, the American Association of Endodontists and the publisher disclaim any and all responsibility or liability for such material.

Before You Begin

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Conflict of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:*Before the accepted manuscript is published in an online issue*: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Reporting clinical trials

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.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright see http://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail will be sent to the

corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

Retained author rights

As an author you (or your employer or institution) retain certain rights; for details you are referred to: http://www.elsevier.com/authorsrights.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (

http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Submit your article

Please submit your article via http://ees.elsevier.com/joe/.

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, ~~I~~n 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: ~~S~~odium 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 ~~w~~as," ~~w~~ere," ~~c~~ould," etc. For example: ~~D~~examethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: ~~O~~ur 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, ~~F~~ormerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to ~~F~~ormerly, 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.

h. Authors should be aware that the JOE uses iThenticate, plagiarism detection software, to ensure originality and integrity of material published in the journal. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by the new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Essential title page information

• ***Title.***Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the**

complete postal address. Contact details must be kept up to date by the corresponding author.

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Structured abstract

A structured abstract, by means of appropriate headings, should provide the context or background for the research and should state its purpose, basic procedures (selection of study subjects or laboratory animals, observational and analytical methods), main findings (giving specific effect sizes and their statistical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations.

Abstract Headings

Introduction, Methods, Results, Conclusions

Keywords

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

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc).

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

Original Research Article Guidelines

Title Page

The title describes the major emphasis of the paper. It must be as short as possible without loss of clarity. Avoid abbreviations in the title because this may lead to imprecise coding by electronic citation programs such as PubMed (eg, use sodium hypochlorite rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at www.icmje.org). Include the manuscript title; the names and affiliations of all authors; and the name, affiliation, and full mailing address (including e-mail) of the corresponding author. This author will be responsible for proofreading page proofs and ordering reprints when applicable. Also highlight the contribution of each author in the cover letter.

Abstract

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

hypothesis is described in the Abstract Introduction. The Abstract describes the new contributions made by this study. The Abstract word limitation and its wide distribution (eg, PubMed) make it challenging to write clearly. This section is written last by many authors. Write the abstract in past tense because the study has been completed. Provide 3-5 keywords.

Introduction

The introduction briefly reviews the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. Clearly describe the purpose of the study, the tested hypothesis, and its scope. Many successful manuscripts require no more than a few paragraphs to accomplish these goals; therefore, do not perform extensive literature review or discuss the results of the study in this section.

Materials and Methods

The Materials and Methods section is intended to permit other investigators to repeat your experiments. There are 4 components to this section: (1) detailed description of the materials used and their components, (2) experimental design, (3) procedures employed, and (4) statistical tests used to analyze the results. Most manuscripts should cite prior studies that used similar methods and succinctly describe the essential aspects used in the present study. A "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then you must carefully describe the method and include validation experiments. If the study used a commercial product, the manuscript must either state that you followed manufacturer's protocol or specify any changes made to the protocol. If the study used an *in vitro* model to simulate a clinical outcome, describe either experiments made to validate the model or previous literature that proved the clinical relevance of the model. The statistical analysis section must describe which tests were used to analyze which dependent measures; *P* values must be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, dropouts from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.

Results

Only experimental results are appropriate in this section; do not include methods, discussion, or conclusions. Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts, and Tables must be cited in the text in numerical order and include a brief description of the major findings. Consider using Supplemental Figures, Tables, or Video clips that will be published online. Supplemental material often is used to provide additional information or control experiments that support the results section (eg, microarray data).

Figures

There are 2 general types of figures: type 1 includes photographs, radiographs, or micrographs; type 2 includes graphs. *Type 1:* Include only essential figures and use composite figures containing several panels of photographs, if possible. Each panel must be clearly identified with a letter (eg, A, B, C), and the parts must be defined in the figure legend. A figure that contains many panels counts as 1 figure. *Type 2:* Graphs (ie, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a

function of an independent measure (usually plotted on the X axis). One example is a graph depicting pain scores over time. Use graphs when the overall trend of the results is more important than the exact numeric values of the results. A graph is a convenient way to report that an ibuprofen-treated group reported less pain than a placebo-treated group over the first 24 hours, but pain reported was the same for both groups over the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.

Tables
Tables are appropriate when it is critical to present exact numeric values; however, not all results need be placed in either a table or figure. Instead of a simple table, the results could state that there was no inhibition of growth from 0.001%-0.03% NaOCl, and a 100% inhibition of growth from 0.03%-3% NaOCl (N=5/group). If the results are not significant, then it is probably not necessary to include the results in either a table or as a figure.

Acknowledgments
All authors must affirm that they have no financial affiliation (eg, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements, or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past 3 years. Disclose any potential conflict of interest. Append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Disclose all sources and attribute all grants, contracts, or donations that funded the study. Specific wording: "The authors deny any conflicts of interest related to this study."

References
The reference style can be learned from reading past issues of *JOE*. References are numbered in order of citation. Place text citation of the reference Arabic number in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

Other Article Types and Guidelines

Manuscripts submitted to *JOE* that are not Original Articles must fall into one of the following categories. Abstract limit: 250 words. Note that word limits, listed by type, do not include figure legends or References. If you are not sure whether your manuscript falls within one of the categories listed or if you would like to request pre-approval to submit additional figures, contact the Editor at *JEndodontics@uthscsa.edu*.

CONSORT Randomized Clinical Trial

Must strictly adhere to the Consolidated Standards of Reporting Trials—CONSORT—minimum guidelines for publication of randomized clinical trials (http://www.consort-statement.org). Word limit: 3500. Headings: Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments. Maximum number of figures: 4. Maximum number of tables: 4.

Review Article

Either narrative articles or systemic reviews/meta-analyses. Case Report/Clinical Techniques articles, even when they include an extensive review of the literature, are categorized as Case Report/Clinical Techniques. Word limit: 3500. Headings: Abstract, Introduction, Discussion, Acknowledgments. Maximum number of figures: 4. Maximum number of tables: 4.

Clinical Research

Prospective or retrospective studies of patients or patient records, research on biopsies excluding the use of human teeth for technique studies. Word limit: 3500. Headings: Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments. Maximum number of figures: 4. Maximum number of tables: 4.

Basic Research—Biology

Animal or culture studies of biological research on physiology, development, stem cell differentiation, inflammation, or pathology. Primary focus is on biology. Word limit: 2500. Headings: Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments. Maximum number of figures: 4. Maximum number of tables: 4.

Basic Research—Technology

Focus primarily on research related to techniques and materials used, or on potential clinical use, in endodontics. Word limit: 2500. Headings: Abstract, Introduction, Material and Methods, Results, Discussion, Acknowledgments. Maximum number of figures: 3. Maximum number of tables: 3.

Case Report/Clinical Techniques

Reports of an unusual clinical case or use of a cutting edge technology in a clinical case. Word limit: 2500. Headings: Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments. Maximum number of figures: 4. Maximum number of tables: 4.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Database linking

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately

• Size the illustrations close to the desired dimensions of the printed version.

• Submit each illustration as a separate file.

A detailed guide on electronic artwork is available on our website: http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.

TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;

• Supply files that are too low in resolution;

• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) in addition to color reproduction in print. For further information on the preparation of electronic artwork, please see

http://www.elsevier.com/artworkinstructions.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

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.

References

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not allowed in the reference list, but they may be mentioned in the text. Citation of a reference as "in press" implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web References

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references are included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style

Text: Indicate references by Arabic numerals in parentheses, numbered in the order in which they appear in the text. *List:* Number the references in the list in the order in which they appear in the text. List 3 authors then et al.

Examples:
Journal article:

1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article.JSciCommun. 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.
Journal abbreviations source

Journal names are abbreviated according to Index medicus.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address

• Full postal address

• Phone numbers

All necessary files have been uploaded, and contain:

• Keywords

• All figure captions

• All tables (including title, description, footnotes)

Further considerations

• Manuscript has been 'spell-checked' and 'grammar-checked'

• References are in the correct format for this journal

• All references mentioned in the Reference list are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Web)

• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print

• If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

AnexoB

unesp 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