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Karine Padoin

EFEITO DA ATIVAÇÃO ULTRASSÔNICA DE NOVOS CIMENTOS ENDODÔNTICOS NA RESISTÊNCIA DE UNIÃO E QUALIDADE DA OBTURAÇÃO DE CANAIS RADICULARES

Santa Maria, RS
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, Ênfase em Endodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial à obtenção do título de **Mestre em Ciências Odontológicas**.

Orientadora: Profª. Drª. Renata Dornelles Morgental

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RESUMO

EFEITO DA ATIVAÇÃO ULTRASSÔNICA DE NOVOS CIMENTOS ENDODÔNTICOS NA RESISTÊNCIA DE UNIÃO E QUALIDADE DA OBTURAÇÃO DE CANAIS RADICULARES

AUTORA: Karine Padoin
ORIENTADORA: Renata Dornelles Morgental

O objetivo deste estudo foi investigar o efeito da ativação ultrassônica (AU) de três cimentos endodônticos na resistência de união à dentina radicular e na qualidade da obturação de canais radiculares. Noventa e seis incisivos bovinos foram selecionados. Após acesso e instrumentação, os canais radiculares foram obturados empregando AH Plus (AP), Sealer Plus (SP) ou Sealer Plus BC (BC), com ou sem AU (n=16/grupo). Posteriormente, duas fatias de 1,5 mm foram obtidas de cada terço radicular. A primeira fatia foi submetida ao teste *push-out* e à análise do modo de falha. Já a segunda fatia foi avaliada em estereomicroscópio para análise da qualidade da obturação, utilizando um sistema de escores que considera a presença de espaços vazios. Os dados foram analisados pelos testes de Kruskal-Wallis, Mann-Whitney e Friedman (α=0.05). Diferenças significativas entre os cimentos e tipos de ativação foram detectadas apenas no terço apical ou quando os dados dos terços radiculares foram agrupados. Quando AU não foi utilizada, SP apresentou maior resistência de união do que AP e BC. Além disso, SP apresentou menores escores de espaços vazios do que BC. AU melhorou a resistência de união quando BC foi empregado, mas não afetou a qualidade da obturação de nenhum cimento. Não houve diferenças significativas entre os cimentos ativados por ultrassom quanto à resistência de união ou qualidade da obturação. Os valores de resistência de união foram semelhantes ao longo da raiz, mas houve uma tendência de mais espaços vazios no terço apical. O modo de falha predominante foi o tipo misto. Pode-se concluir que AU foi eficaz no aumento da resistência de união do cimento biocerâmico (BC), mas não melhorou sua qualidade de obturação. Os cimentos à base de resina epóxica (AP e SP) não foram afetados pela AU.

Palavras-chave: Cimentos Dentários. Endodontia. Obturação do Canal Radicular. Tratamento do Canal Radicular. Ultrassom.

ABSTRACT

EFFECT OF ULTRASONIC ACTIVATION OF NEW ENDODONTICS SEALERS IN THE BOND STRENGTH AND ROOT CANAL FILLING QUALITY

AUTHOR: KARINE PADOIN
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The aim of this study was to investigate the effect of ultrasonic activation (UA) of three endodontic sealers on the bond strength to root dentin and root canal filling quality. Ninety-six bovine incisors were selected. After access and instrumentation, root canal filling was carried out using AH Plus (AP), Sealer Plus (SP) or Sealer Plus BC (BC), with or without UA (n=16/group). Then, two 1.5-mm slices were obtained from each root third. The first slice was subjected to push-out testing and failure mode analysis. The second slice was observed under a stereomicroscope for filling quality assessment, using a void score system. Data were analyzed by Kruskal-Wallis, Mann-Whitney and Friedman tests (α=0.05). Significant differences between sealers and types of activation were detected only in the apical third or when root thirds data were pooled. When no UA was applied, SP had higher bond strength than AP and BC. Also, SP presented lower void scores than BC. UA improved bond strength when BC was used, but did not affect the filling quality of any sealer. There were no significant differences between ultrasonically activated sealers, regarding bond strength and filling quality. Bond strength values were similar along the root depth, but there was a tendency to more voids in the apical third. The predominant failure mode was the mixed type. In conclusion, UA was effective in increasing the bond strength of the bioceramic sealer (BC), but did not improve its filling quality. Epoxy resin-based sealers (AP and SP) were not affected by UA.

Key-words: Dental Cements. Endodontics. Root Canal Obturation. Root Canal Therapy. Ultrasonics.

SUMÁRIO

1 INTRODUÇÃO

O objetivo da obturação do sistema de canais radiculares é preenchê-lo tridimensionalmente e vedá-lo de forma eficaz, após sua limpeza e modelagem (ØRSTAVIK, 2014). Diversos estudos têm demonstrado estreita relação entre a qualidade técnica da obturação e o sucesso do tratamento endodôntico (GILLEN et al., 2011; RAMEY; YACCINO; WEALLEANS, 2017; TAVARES et al., 2009). A obtenção de uma obturação adequada envolve vários desafios, incluindo as dificuldades relacionadas à aderência dos materiais obturadores às paredes dentinárias (IGLECIAS et al., 2017; ØRSTAVIK, 2014).

Vários cimentos endodônticos foram desenvolvidos ao longo dos anos para serem empregados em associação com a guta-percha, a qual constitui o núcleo sólido da obturação (ØRSTAVIK, 2005). As características ideais desses cimentos incluem, além das propriedades físico-químicas indispensáveis para uso clínico (ZHOU et al., 2013), boas propriedades adesivas à dentina radicular (GATEWOOD, 2007). Tais propriedades são importantes tanto em uma situação estática quanto dinâmica. Em uma situação estática, o cimento deveria idealmente eliminar qualquer espaço que permita a percolação de fluidos entre a obturação e a parede do canal. Já em uma situação dinâmica, é necessário que haja resistência do material ao deslocamento durante a função subsequente (ØRSTAVIK; ERIKSEN; BEYER-OLSEN, 1983; UNGOR; ONAY; ORUCOGLU, 2006). É importante ressaltar que a união entre os materiais obturadores e as paredes dentinárias ocorre por meio de retenção friccional e adesão micromecânica (MADHURI et al., 2016; MOINZADEH; JONGSMA; WESSELINK, 2015).

Neste contexto, o teste *push-out* vem sendo extensamente utilizado, empregando dentina humana ou bovina como substrato (BARBIZAM et al., 2011; HOPPE et al., 2015; MADHURI et al., 2016; MISHRA et al., 2017; SAGSEN et al., 2011; TRINDADE et al., 2018). *Push-out* é um teste mecânico em que uma carga é aplicada longitudinalmente ao eixo da raiz até que o material seja desalojado, estabelecendo assim sua resistência de união ou resistência ao deslocamento (COLLARES et al., 2016). Tal teste baseia-se no esforço de cisalhamento na interface cimento-dentina (VAN MEERBEEK et al., 2003). Apesar de não replicar completamente o comportamento clínico dos cimentos endodônticos, o teste *push-*

out fornece informações valiosas na comparação entre diferentes cimentos ou técnicas de obturação (MOINZADEH; JONGSMA; WESSELINK, 2015), permitindo a obtenção de vários espécimes de uma mesma raiz e a avaliação de diferenças regionais entre os terços do canal radicular (GORACCI et al., 2004).

As propriedades físico-químicas dos cimentos endodônticos também podem influenciar a qualidade da obturação e potencialmente o sucesso do tratamento endodôntico (MUTAL; GANI, 2005). Espaços vazios presentes na massa obturadora podem conectar-se com canais laterais, canais acessórios e túbulos dentinários, os quais podem abrigar microrganismos (LOVE; JENKINSON, 2002). Sendo assim, um denso preenchimento do canal radicular poderia impedir a sobrevivência de microrganismos e a difusão de seus produtos em direção ao terço apical da raiz, o que influenciaria a saúde dos tecidos perirradiculares (HARGREAVES; COHEN, 2011).

Há muitas décadas as resinas sintéticas têm sido utilizadas como materiais obturadores de canais radiculares, ganhando popularidade a partir do desenvolvimento dos materiais da série AH, há mais de 50 anos (MURUZÁBAL; ERAUSQUIN, 1966). O cimento AH Plus (Dentsply DeTrey Gmbh, Konstanz, Alemanha), uma mistura de epóxi-aminas, é um dos produtos resultantes deste desenvolvimento, sendo utilizado na prática clínica mundialmente e também como “padrão-ouro” em pesquisas científicas (HERGT et al., 2015). O AH Plus é empregado como parâmetro de comparação para novos cimentos, em virtude de suas excelentes propriedades físico-químicas (ZHOU et al., 2013), biológicas (LEONARDO et al., 1999; SCARPARO; GRECCA; FACHIN, 2009), antimicrobianas (HEYDER et al., 2013; SALEH et al., 2004) e adesivas (WIESSE et al., 2018).

Recentemente, foi lançado no mercado nacional o cimento Sealer Plus (MK Life, Porto Alegre, RS, Brasil), também à base de resina epóxica, disponível em seringa de corpo duplo, a qual dispensa porções iguais das pastas base e catalisadora. No estudo conduzido por Vertuan et al. (2018), quando comparado ao AH Plus, tal cimento demonstrou solubilidade, escoamento e pH semelhantes, porém menor radiopacidade e tempo de presa. No entanto, ambos os cimentos apresentaram propriedades físico-químicas compatíveis com as recomendações da norma ISO 6876:2012 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2012).

Quanto às propriedades biológicas do cimento Sealer Plus, o estudo de Cintra et al. (2017) demonstrou menor citotoxicidade e biocompatibilidade semelhante ao AH Plus, sendo esta última avaliada em tecido subcutâneo de ratos. O Sealer Plus também produziu menor resposta inflamatória quando comparado a um cimento à base de óxido de zinco e eugenol. Tais autores reforçaram que as propriedades biológicas favoráveis do Sealer Plus devem estar relacionadas à presença de hidróxido de cálcio na sua composição. Apesar desta caracterização físico-química e biológica, ainda faltam estudos científicos avaliando as propriedades adesivas desse material à dentina radicular.

Cimentos endodônticos biocerâmicos pré-misturados foram introduzidos no mercado na última década e têm recebido grande atenção da comunidade científica em função de suas ótimas propriedades biológicas e seu potencial para biomineralizacão (CHANG et al., 2014; GÜVEN et al., 2013; SILVA ALMEIDA et al., 2017). Nessa classe, encontram-se os cimentos iRoot SP (Innovative Bioceramix, Vancouver, BC, Canadá) e Endosequence BC Sealer (Brasseler USA, Savannah, GA, EUA). Suas composições incluem silicato de cálcio, fosfato de cálcio, hidróxido de cálcio, agentes de preenchimento e espessantes, além de óxido de zircônia como radiopacificador (AL-HADDAD; CHE AB AZIZ, 2016). Além da comprovada biocompatibilidade (BÓSIO et al., 2014; ZHANG; LI; PENG, 2010), outra vantagem dos cimentos biocerâmicos seria a formação de hidroxiapatita durante o processo de presa, devido à presença do silicato de cálcio, criando uma possível ligação química entre a dentina e o material obturador (SHOKOUHINEJAD et al., 2013).

O cimento biocerâmico Sealer Plus BC (MK Life, Porto Alegre, RS, Brasil) foi lançado recentemente, com composição semelhante aos materiais supracitados, produzidos por empresas estrangeiras. O fabricante alega uma capacidade de vedamento notável, em virtude da adesão química à estrutura dental e da expansão higroscópica que ocorreria durante a presa (MK LIFE, 2018). Porém, ao observar a literatura científica vigente, não são encontrados estudos avaliando as propriedades adesivas desse cimento à dentina radicular.

Aparelhos ultrassônicos vêm sendo utilizados na odontologia há muitos anos, sendo referenciados pela primeira vez por Catuma, na década de 50, para uso em preparos cavitários (WALMSLEY, 1988). Desde então, sua aplicação em diversas especialidades odontológicas vem aumentando de forma constante, sendo atualmente empregados em odontologia restauradora, periodontia, endodontia, ortodontia, cirurgia bucomaxilofacial e diagnóstico oral (CHEN et al., 2013).

Na prática endodôntica, o maior destaque do ultrassom está em seu uso na agitação de soluções irrigadoras, sem instrumentação ultrassônica simultânea, técnica conhecida como irrigação ultrassônica passiva (*passive ultrasonic irrigation* - PUI) (VAN DER SLUIS et al., 2007). Tal técnica tem sido descrita como um excelente auxiliar no processo de limpeza do sistema de canais radiculares, em virtude dos fenômenos de transmissão acústica e cavitação (PLOTINO et al., 2007). Estudos prévios demonstram que a técnica promove maior remoção de debris orgânicos e inorgânicos (SABINS; JOHNSON; HELLSTEIN, 2003; VIVAN et al., 2016), resultando em paredes radiculares mais limpas (KOÇAK et al., 2017). Assim, o emprego da PUI pode potencializar a ação antimicrobiana da medicação intracanal (ARIAS et al., 2016), além de proporcionar maior penetração do cimento obturador nos túbulos dentinários (AKSEL et al., 2017).

A aplicação do ultrassom para ativação de cimentos endodônticos também já foi sugerida, com o intuito de melhorar a qualidade da obturação dos canais radiculares (GUIMARÃES et al., 2014). Pesquisas recentes mostram que a ativação ultrassônica de alguns cimentos pode proporcionar: maior resistência de união à dentina (WIESSE et al., 2018), maior penetração em canais laterais (ARSLAN; ABBAS; KARATAS, 2016) e túbulos dentinários (NIKHIL; BANSAL; SAWANI, 2015), além de reduzir a presença de áreas não obturadas e aumentar a penetração dos cimentos em regiões de istmo (ALCALDE et al., 2017). Porém, não se sabe se esse efeito benéfico do ultrassom também é observado com os novos cimentos resinosos e biocerâmicos disponíveis no mercado.

O presente trabalho será apresentado em forma de artigo científico, intitulado “Effect of ultrasonic activation of new endodontics sealers in the bond strength and root canal filling quality”, tendo como objetivo avaliar o efeito da ativação ultrassônica de três cimentos endodônticos, dois à base de resina epóxica (AH Plus e Sealer Plus) e um biocerâmico (Sealer Plus BC), na resistência de união à dentina radicular bovina e na qualidade da obturação do canal radicular.

2 ARTIGO – EFFECT OF ULTRASONIC ACTIVATION OF NEW ENDODONTICS SEALERS IN THE BOND STRENGTH AND ROOT CANAL FILLING QUALITY

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EFFECT OF ULTRASONIC ACTIVATION OF NEW ENDODONTICS SEALERS IN THE BOND STRENGTH AND ROOT CANAL FILLING QUALITY

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Abstract

Introduction: The aim of this study was to investigate the effect of ultrasonic activation (UA) of three endodontic sealers on the bond strength to root dentin and root canal filling quality. **Methods:** Ninety-six bovine incisors were selected. After access and instrumentation, root canal filling was carried out using AH Plus (AP), Sealer Plus (SP) or Sealer Plus BC (BC), with or without UA (n=16/group). Then, two 1.5-mm slices were obtained from each root third. The first slice was subjected to push-out testing and failure mode analysis. The second slice was observed under a stereomicroscope for filling quality assessment, using a void score system. Data were analyzed by Kruskal-Wallis, Mann-Whitney and Friedman tests (α=0.05). **Results:** Significant differences between sealers and types of activation were detected only in the apical third or when root thirds data were pooled. When no UA was applied, SP had higher bond strength than AP and BC. Also, SP presented lower void scores than BC. UA improved bond strength when BC was used, but did not affect the filling quality of any sealer. There were no significant differences between ultrasonically activated sealers, regarding bond strength and filling quality. Bond strength values were similar along the root depth, but there was a tendency to more voids in the apical third. The predominant failure mode was the mixed type. **Conclusions:** UA was effective in increasing the bond strength of the bioceramic sealer (BC), but did not improve its filling quality. Epoxy resin-based sealers (AP and SP) were not affected by UA.

Key Words: Bioceramic sealer, push-out bond strength, root canal filling quality, ultrasonic activation.

Introduction

Ultrasound was first introduced to endodontics in the 1950s and has been used in several endodontic procedures, ranging from access cavity refinement to apical surgery (1). Currently, its main use is for the agitation of irrigating solutions, without simultaneous ultrasonic instrumentation, the so-called passive ultrasonic irrigation (PUI) (2). It promotes acoustic streaming and cavitation forces that lead to greater removal of organic and inorganic debris from the root canal system (1) resulting in better cleaning of the dentinal walls (3). Therefore, PUI may enhance the antimicrobial action of intracanal dressings (4) as well as improve the penetration of endodontic sealers in dentinal tubules (5).

The application of ultrasonic devices in obturation procedures has also been proposed, aiming to improve root canal filling quality (6,7). Previous studies have shown that the ultrasonic activation of endodontic sealers may promote: higher bond strength to root dentin (8), greater penetration of sealers into lateral canals (9), isthmuses (10) and dentinal tubules (9), in addition to better interfacial adaptation between the filling material and root canal walls (6,8).

However, the chemical composition and viscosity of endodontic sealers vary greatly, which may interfere with ultrasonic activation (8), producing different results. AH Plus (Dentsply DeTrey Gmbh, Konstanz, Germany), an epoxy resin-based sealer, has been extensively investigated and it is considered the ‘gold-standard’ among endodontic sealers due to its excellent physicochemical properties (11), biological behavior (12) and adhesion to dentin (8).

New endodontic sealers have been introduced to the market, such as Sealer Plus (MK Life, Porto Alegre, RS, Brazil). It is another epoxy resin-based sealer with reports in the literature about its physicochemical (13) and biological (14) properties. When compared to AH Plus, it showed similar solubility, flow and pH, but lower radiopacity and setting time (13). Yet, both sealers presented physicochemical properties in accordance with ISO 6876:2012 recommendations (15). Unlike AH Plus, Sealer Plus contains calcium hydroxide, which may explain its low cytotoxicity and high biocompatibility (14).

Pre-mixed bioceramic endodontic sealers have received great attention from the scientific community in the last years due to their biological properties and potential for biomineralization (16). A new bioceramic sealer is commercially available and has not had its properties studied, Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil). It contains calcium silicate, calcium hydroxide, zirconium oxide and propylene glycol (17). As other pre-mixed calcium silicate-based sealers, it relies on water within the dentinal tubules to set (18). This class of sealers has the potential to form hydroxyapatite during the setting process and ultimately generate a chemical bond between the filling material and dentin (19). The behavior of these new sealers under ultrasonic activation is still unknown.

Based on these observations, the aim of this study was to evaluated the effect of ultrasonic activation of two epoxy resin-based sealers (AH Plus and Sealer Plus) and one bioceramic sealer (Sealer Plus BC) on their bond strength to root dentin and root canal filling quality. The null hypothesis tested was that ultrasonic activation of sealers would not influence bond strength and filling quality.

Materials and Methods

Sample size calculation

Sample size was calculated using the parameters described by Wiesse et al. (8): minimum difference between groups of 0.60 MPa; standard deviation of 0.45; power of 80%; alpha 0.05; for 6 treatments (BioEstat 5.0 software; Instituto Mamirauá, Belém, PR, Brazil). The estimated minimum sample size was found to be 16 per group.

Sample selection and preparation

Ninety-six bovine incisors from animals killed for commercial reasons (ANEXO B) were used. Teeth were cleaned with periodontal curettes (Golgran, São Paulo, SP, Brazil) and stored in 0.5% chloramine-T solution (Sigma-Aldrich Brasil LTDA, Duque de Caxias, RJ, Brazil) at 4ºC for a week (20). Specimens were observed under a digital stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany) at 8× magnification and those with cracks, incomplete root formation or other structural anomalies were excluded.

Teeth were decoronated with carborundum discs (KG Sorensen, Barueri, SP, Brazil) to standardize a remaining root length of 20 mm. Next, roots were selected according to the anatomical dimensions of a size 70 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) to reduce the size variation between root canals.

Initially, root canals were irrigated with 5 mL of 2.5% sodium hypochlorite (NaOCl; Biodinâmica, Ibiporã, PR, Brazil) and the working length (WL) was established by measuring the penetration of a size 15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland), introduced passively until reaching the apical foramen and then subtracting 1 mm. Root canal preparation was carried out by a crown-down technique, using size 6 and 5 Gates-Glidden drills (Dentsply-Maillefer, Ballaigues, Switzerland) in the coronal and middle thirds, respectively. The apical third was prepared by hand stainless steel instruments, from a size 70 to a size 100 K-file (Dentsply-Maillefer, Ballaigues, Switzerland).

All procedures were performed under copious irrigation, using 2 ml of 2.5% NaOCl at each instrument change. After chemomechanical preparation, root canals were irrigated with 5 ml of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 5 minutes to remove the smear layer and then irrigated with 10 ml of distilled water (8). Root canals were dried with absorbent paper points (Dentsply Brazil, Petrópolis, RJ, Brazil) and size 100/.02 taper gutta-percha points (Dentsply Brazil, Petrópolis, RJ, Brazil) were tested for tug-back at the WL and confirmed radiographically.

Root canal filling

Specimens were randomly distributed into six experimental groups, using www.randomization.com, according to the endodontic sealer (Supplemental Table S1) and type of sealer activation:

- AP: AH Plus without activation;
- APU: AH Plus with ultrasonic activation;
- SP: Sealer Plus without activation;
- SPU: Sealer Plus with ultrasonic activation;
- BC: Sealer Plus BC without activation;
- BCU: Sealer Plus BC with ultrasonic activation.

Sealers were manipulated according to the manufacturer's instructions and inserted into the canals with a size 4 Lentulo spiral (Dentsply-Maillefer, Ballaigues, Switzerland) at low speed for 5 seconds. This procedure was repeated until the root canal walls were completely cover by sealer. In the groups of ultrasonically activated sealers (APU, SPU and BCU), activation was performed immediately after sealer placement, using an E1 Irrisonic tip (Helse Ultrasonic, Ribeirão Preto, SP, Brazil) attached to an ultrasonic device (Sonic Laxis BP LED, Schuster, Santa Maria, RS, Brazil), 2 mm short of the WL, at 20% power level. As the ultrasonic insert oscillates in a single plane, it was activated for 20 seconds in the mesiodistal direction and another 20 seconds in the buccolingual direction (10). Gentle brushing movements were performed against the root canal walls.

Next, in all groups, a size 100/.02 taper gutta-percha point was inserted into the full WL and the root canal obturation was complemented by the lateral condensation technique with a D-size finger spreader (Dentsply-Maillefer, Ballaigues, Switzerland) and accessory gutta-percha points (Dentsply-Maillefer, Ballaigues, Switzerland). After radiographic confirmation of complete root canal filling, the excess of material in the coronal portion was removed by a heated plugger (Golgran, São Paulo, SP, Brazil) and then the specimens was sealed with a temporary restorative material (Coltosol; Coltene, Altstätten, Switzerland). Then, specimens were stored at 37ºC and 100% humidity for 24 hours to allow the sealers to set (8).

Push-out bond strength test

Specimens were transversally sectioned using a precision cutting machine (Isomet; Extec Corp, Enfield, CT, USA) set at 300 rpm and equipped with a double-sided diamond disc (Buehler, Lake Bluff, IL, USA). To facilitate their fixation in the machine, the coronal portion was included in self-cured acrylic resin (Clássico, Campo Lindo Paulista, SP, Brazil). The most coronal and apical portions of each specimen were discarded and six 1.5 mm-thick (± 0.3 mm) slices were produced from each root, two per root third (coronal, middle and apical).

The push-out test was performed in a universal testing machine (EMIC DL-2000; EMIC, São José dos Pinhais, PR, Brazil). Root slices were positioned in the machine with their coronal surfaces facing down and then submitted to compression loading using a metallic plunger with a 0.8 mm-diameter tip touching the root canal filling center. Loading forces were introduced from an apical to coronal direction (1 mm/min speed) and the bond strength (σ) was obtained in megapascal (MPa) as previously reported (21). The following formula was applied: $\sigma = F/A$, where F = load for filling dislodgement (N) and A = adhesion area (mm^2). To determine A, a formula was used to calculate the lateral area of a straight circular cone with parallel bases: $A = 2\pi g (R_1 + R_2)$, where $\pi = 3.14$, g = inclined height, R_1 = smaller base radius, R_2

= larger base radius. To determine g, the following calculation was used: $g^2 = (H^2 + [R_1 - R_2]^2)$, where H = section height. R_1 and R_2 were obtained by measuring the internal diameters of the smallest and largest base, respectively, corresponding to the internal diameter of the root canal walls. H, R_1 and R_2 were measured after the test with a digital caliper (Mitutoyo, Suzano, SP, Brazil).

Failure mode analysis

After the push-out test, slices were analyzed by a single blinded and calibrated (*kappa*=0.83) examiner using a digital stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany) at 25× magnification to determine the failure pattern. Failures were classified as: adhesive, when the sealer was completely separated from the dentin (surface without sealer), cohesive when the failure occurred within the filling material (dentin surface completely covered by sealer), and mixed when a mixture of adhesive and cohesive modes occurred (dentin surface partially covered by sealer) (22).

Filling quality assessment

The second slices obtained from each root third were used to investigate the filling quality promoted by the different sealers and types of activation. They were observed under a digital stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany) at 25× magnification and pictures of each slice were taken. Digital images were evaluated to estimate the presence, number and diameter of voids within the filling material, using a four-point scoring system (7). For void diameter calculation, the ImageJ 1.46 software (National Institutes of Health, Bethesda, MD, USA) was used with standardized 75% zoom.

Filling quality was assessed by a single blinded and calibrated (*weighted kappa*=0.84) examiner. The following scores were considered: 1) well-condensed filling material with only a few small air bubbles (< 0.1 mm in diameter); 2) imperfectly condensed filling with some small air bubbles (more than 3 defects) or medium-sized air bubbles (0.1 to 0.2 mm in diameter); 3) inadequately condensed filling with several small air bubbles (more than 5 defects) or large air bubbles (> 0.2 mm in diameter); 4) poorly condensed filling, presenting many small air bubbles (more than 7 defects) or void space connecting separate root canal walls (7).

Statistical analysis

Bond strength data were not normally distributed and then were analyzed by non-parametric tests. Sealers, types of activation and root thirds were compared by Kruskal-Wallis, Mann-Whitney and Friedman tests, respectively. Void scores were analyzed in the same manner. The significance level was set at 5% (SPSS Statistics 20 software; IBM SPSS Inc., Chicago, IL, USA).

Results

Push-out bond strength

Bond strength results are summarized in Table 1. For non-ultrasonically activated sealers, SP had higher bond strength than BC in the apical third (P=0.001) and overall (P=0.001). SP also showed higher bond strength values than AP in the apical third (P=0.021). There was no significant difference between ultrasonically activated sealers in any root segment (P>0.05).

Ultrasonic activation of BC resulted in higher bond strength than no activation in the apical third (P=0.042) and overall (P=0.011). Ultrasond did not affect the bond strength of AP and SP. No significant difference was detected between root thirds for any sealer, regardless of ultrasonic activation (P>0.05).

Failure mode

Failure mode distribution (%) in the specimens is displayed in Figure 2. There was a higher frequency of mixed failures in all experimental groups and root thirds.

Filling quality

The results of filling quality, represented by void scores, are expressed in Table 2. In the groups without activation, BC showed higher void scores than SP in the apical third (P=0.014) and overall (P=0.008). In the ultrasonically activated groups, no significant difference was detected in any root segment (P>0.05). Comparison between root thirds revealed a tendency to higher void scores in the apical segment for all experimental groups. AP, APU and BC showed significantly higher scores in the apical third than in the coronal third (P=0.008; P=0.011; P=0.001, respectively). AP also presented higher scores in the middle third compared to the coronal third (P=0.021).

Discussion

New root canal filling materials are continuously released on the market and incorporated into the clinical endodontic arsenal. However, the choice of dental materials should be guided by scientific evidence based on laboratory and clinical studies. To the best of our knowledge, this is the first study to evaluate the bond strength and filling quality promoted by SP and BC, and the effect of ultrasonic activation on their properties. AP was used as the reference sealer for comparison, as described by several other authors (6-8,11,16). The null hypothesis was partially rejected because ultrasonic activation improved the bond strength of BC, but did not affect the filling quality of any sealer.

Regarding bond strength, comparison between groups without ultrasonic activation revealed no significant differences in the coronal and middle segments, but SP presented higher values than BC in the apical third and when data of all root thirds were pooled. Previous studies have explained the good bond strength performance of epoxy resin–based sealers by the ability to form a covalent bond to any exposed amino groups in dentin collagen, when the epoxide ring opens (23). Interestingly, in the apical third, SP was also superior to AP. Both are epoxy resin-based sealers, but have different compositions (13) and probably different viscosities. Possible differences in the proportion of resinous components (bisphenol-A and bisphenol-F epoxy resins) and the presence of calcium hydroxide in SP could explain these findings. Duarte and Moraes (24) have shown that the addition of calcium hydroxide leads to an improved sealing capacity of AP, as determined by dye infiltration. However, it is important to note that microleakage methodologies have been severely criticized (25) and that no direct association between apical seal and bond strength can be established (26).

In this study, a significant improvement in the bond strength of BC was verify when the sealer was ultrasonically activated, which is in agreement with previous findings (8). This fact could be explained by the heat generated during the process, reducing sealer viscosity, combined with the transmission of acoustic streaming energy produced by the ultrasonic tip, forcing the sealer against the canal walls (8-10). There was also an increase in bond strength of AP and SP, but it was not statistically significant. Despite using the ultrasonic insert with brushing movements, the wide diameter of the root canals of bovine teeth may be responsible for the lack of significant difference between activated and non-activated resinous sealers. The possibility of a significant improvement in narrower canals cannot be discarded. It is noteworthy that Wiesse et al. (8) found improvement when AP was ultrasonically activated, but the authors used root canals of human teeth with more restricted apical sizes.

The absence of significant differences between root segments regarding bond strength, as observed here, has already been reported (27). Controversially, other studies have shown lower bond strength in the apical third in comparison to that of the coronal third (28). In this study, endodontic sealers were introduced into the canals by a Lentulo spiral, which may alloy a more homogeneous distribution of the sealer up to the apex (29). Moreover, according to Dash et al., (30) the sealer achieves greater penetrability in dentinal tubules when applied by this instrument.

The mixed failure, where adhesive and cohesive failures coexist, was the most common failure mode induced by the push-out test, representing more than 60% of the specimens in all experimental groups and root thirds. These findings are in accordance with past studies (8,22). However, there is no consensus in the endodontic literature with respect to the failure pattern observed with bioceramic and resinous sealers (8,18,22,23,27).

One of the ways to analyze root canal filling quality is through its visual observation. A recent study compared the filling quality promoted by different endodontic sealers using micro-computed tomography (micro-CT), followed by stereomicroscopic observation of root sections (7). No significant difference was found between groups when evaluated by micro-CT, whereas in the stereomicroscopic analysis, a pre-mixed bioceramic sealer (Endoseal MTA; Maruchi, Wonju, Korea) showed greater number of voids than AP. The authors speculated that micro-CT might be less sensitive compared with the sectioning method in terms of void detection, since sealers are considerably radiopaque and this may impair the micro-CT detection of small voids within the bulk of the root filling.

In the present investigation, the second slice of each root third was examined under a stereomicroscope and scored, as described in the aforementioned study (7). In groups without ultrasonic activation, BC presented significantly higher void scores than SP in the apical third and when data of root thirds were polled. On the other hand, BC produced similar void scores compared with AP, regardless of ultrasonic activation. Previous studies also found that pre-mixed bioceramic sealers and AP promote similar filling quality (31,32).

Unlike bond strength results, filling quality was not improved by ultrasonic activation in this study. Similarly, Guimarães et al. (6) evaluated four epoxy resin-based sealers and did not detect differences in void percentage when they were ultrasonically activated, despite observing greater penetration of sealers in dentinal tubules, which was not evaluated here. Kim et al. (7) found lower void scores when ultrasound was applied, but they used a gutta-percha cone-mediated ultrasonic activation, in which the ultrasonic tip did not contact the sealer, but a cotton plier that held the gutta-percha cone.

Ultrasonic activation of sealers did not seem to influence the presence of voids, which probably is more related to the inability of the lateral condensation technique to allow a dense and homogeneous obturation (6). In this study, ultrasound only acted in the adaptation of the sealer to the canal walls, before starting lateral condensation procedures. Better outcomes could be obtained if ultrasound had been used to activate the spreader, while inserting accessory gutta-percha points into the canal, as described by other authors (1). Furthermore, it can the hypothesized that increasing ultrasonic power would improve filling quality. However, it would also increase the risk of fracture of the ultrasonic insert. The power recommended for this purpose in previous studies ranges from 10% to 50% (6,8,30).

When root thirds were compared, there was a tendency to lower void scores in the coronal third, increasing towards the apex. Significant differences were observed for AP, APU and BC. The apical third is the most critical and complex area of the root canal system for both instrumentation and obturation (33). Voids and gaps in this region may be connected with dentinal tubules, accessory canals or other ramifications that may harbor microorganisms (34). It is well-know that an intraradicular infection persisting in the apical root third is the main cause of endodontic treatment failure (35).

Although carefully delineated, this laboratory study can only estimate an increase or reduction in immediate bond strength performance and filling quality promoted by the tested sealers, which should be complemented by further long-term in vitro studies and then confirmed by randomized clinical trials to assess the clinical behavior of such materials.

In conclusion, within the limits of this study, SP showed higher bond strength and less voids than BC in the apical third and when data of root thirds were pooled. SP also presented higher bond strength compared with AH Plus in the apical third. The bond strength and filling quality promoted by both epoxy resin-based sealers were not influenced by ultrasonic activation. However, the ultrasonic activation of BC increased its adhesive property and all ultrasonically activated sealers had similar performance. When root thirds were compared, the bond strength was similar, but there was a tendency to worse filling quality, with more voids, in the apical segment.

Acknowledgments

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

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Figures

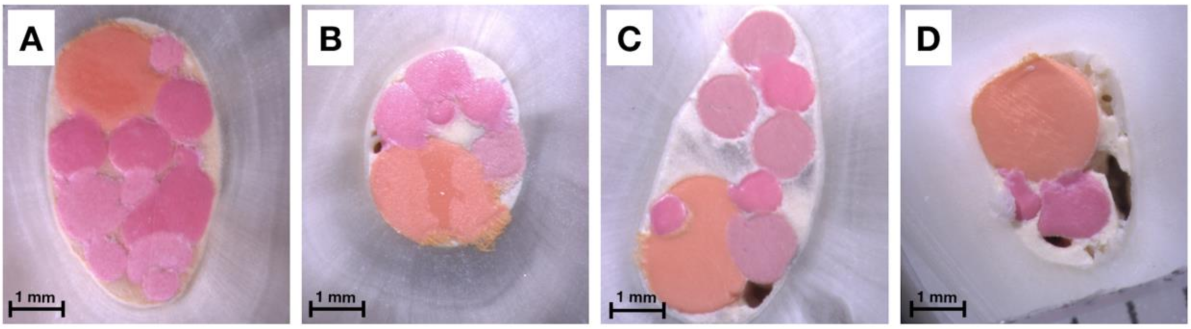

Figure 1. Representative stereomicroscopic images from slices of the middle third (A, B, C, D) at 25× magnification. Score 1, Sealer Plus group (A); Score 2, Sealer Plus ultrasonically activated group (B); Score 3, AH Plus ultrasonically activated group (C); Score 4, AH Plus group (D).

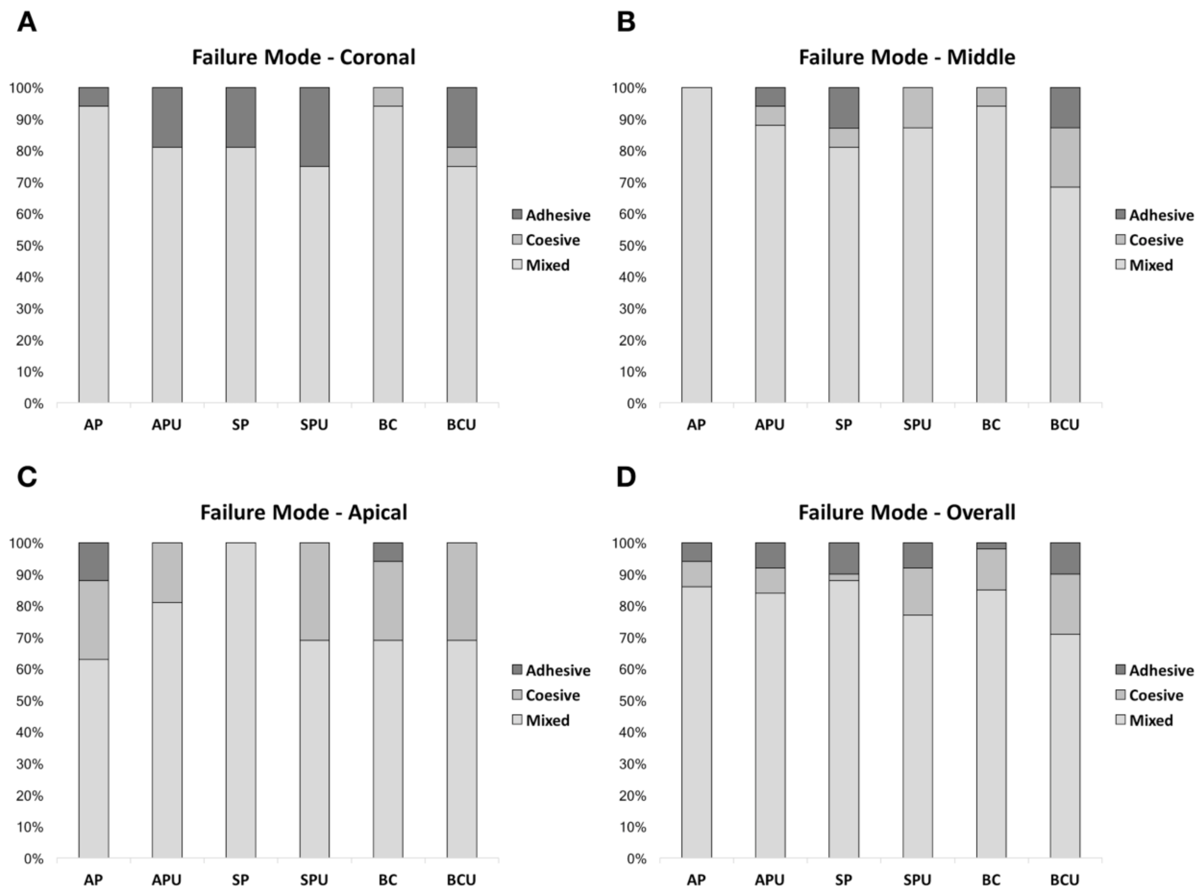

Figure 2. Failure mode distribution (%) according to sealer, type of activation and root third. AP: AH Plus; APU: AH Plus ultrasonically activated; SP: Sealer Plus; SPU: Sealer Plus ultrasonically activated; BC: Sealer Plus BC; BCU: Sealer Plus BC ultrasonically activated.

Tables

Supplemental Table S1. Composition of endodontic sealers and their manufacturers.

Sealer	Composition	Manufacturer
AH Plus	Paste A: bisphenol-A epoxy resin; bisphenol-F epoxy resin; calcium tungstate; zirconium oxide; silica and iron oxide.	Dentsply, DeTrey GmbH, Konstanz, Germany
	Paste B: adamantine amine; n, n ''-dibenzyl-5-oxanone diamine-1,9; TCD-diamine; calcium tungstate; zirconium oxide; silica and silicone oil.	
Sealer Plus	Base paste: bisphenol-A-coepichlorohydrin; bisphenol-F epoxy resin; zirconium oxide; silicon and siloxanes; iron oxide; calcium hydroxide.	MK Life, Porto Alegre, RS, Brazil
	Catalytic paste: hexamethylenetetramine; zirconium oxide; silicon and siloxanes; calcium hydroxide; tungstate calcium.	
Sealer Plus BC	Zirconium oxide; tri-calcium silicate; di-calcium silicate; calcium hydroxide; propylene glycol.	MK Life, Porto Alegre, RS, Brazil

Table 1. Push-out bond strength (MPa) according to sealer, type of activation and root third. Values were expressed in mean and standard deviation.

		Type of activation					
		No activation Root canal sealer			Ultrasonic activation Root canal sealer		
Root third	N	AP	SP	BC	APU	SPU	BCU
Coronal	16	2.20 ± 0.85^{Aa}	1.70 ± 0.46^{Aa}	1.70 ± 0.29^{Aa}	2.11 ± 0.40^{Aa}	2.16 ± 0.83^{Aa}	1.94 ± 0.35^{Aa}
Middle	16	1.80 ± 0.67^{Aa}	2.08 ± 0.44^{Aa}	1.67 ± 0.44^{Aa}	2.12 ± 0.62^{Aa}	2.06 ± 0.31^{Aa}	1.75 ± 0.41^{Aa}
Apical	16	1.59 ± 0.83^{Ba}	2.29 ± 0.54^{Aa}	1.61 ± 0.52^{Bb}	1.86 ± 0.65^{Aa}	2.53 ± 0.65^{Aa}	2.34 ± 1.25^{Aa}
Overall	48	1.86 ± 0.81^{Aba}	2.02 ± 0.53^{Aa}	1.66 ± 0.41^{Bb}	2.03 ± 0.57^{Aa}	2.25 ± 0.65^{Aa}	2.01 ± 0.80^{Aa}

AP: AH Plus; SP: Sealer Plus; BC: Sealer Plus BC; APU: AH Plus ultrasonically activated; SPU: Sealer Plus ultrasonically activated; BCU: Sealer Plus BC ultrasonically activated. Distinct uppercase letters indicate statistically significant difference between sealers (rows), while keeping type of activation and root third unchanged (P<0.05). Distinct lowercase letters indicate statistically significant difference between types of activation (rows), while keeping sealer and root third unchanged (P<0.05). No statistically significant difference was detected between root thirds (column) for any sealer and type of activation (P>0.05).

Table 2. Void scores according to sealer, type of activation and root third.

			Root third																				
			Coronal					Middle					Apical						Overall				
			Scores					Scores					Scores						Scores				
Sealer	Type of activation	N	1	2	3	4	Median	1	2	3	4	Median	1	2	3	4	Median	N	1	2	3	4	Median
AH Plus	NA	16	9	5	2	0	1^{Ab}	3	6	3	4	2^{Aa}	4	3	8	1	3^{ABab}	48	16	14	13	5	2^{AB}
	UA	16	13	1	2	0	1^{Ab}	8	0	6	2	2^{Aab}	5	0	10	1	3^{Aa}	48	26	1	18	3	1^{A}
Sealer Plus	NA	16	9	4	3	0	1^{Aa}	8	3	4	1	1.5^{Aa}	7	2	7	0	2^{Ba}	48	24	9	14	1	1.5^{B}
	UA	16	8	5	3	0	1.5^{Aa}	7	6	3	0	2^{Aa}	6	3	6	1	2^{Aa}	48	21	14	12	1	2^{A}
Sealer Plus BC	NA	16	11	3	2	0	1^{Ab}	3	2	10	1	3^{Aab}	0	3	10	3	3^{Aa}	48	14	8	22	4	3^{A}
	UA	16	7	7	2	0	2^{Aa}	7	2	6	1	2^{Aa}	5	2	7	2	3^{Aa}	48	19	11	15	3	2^{A}

NA: No activation; UA: Ultrasonic activation. Distinct uppercase letters indicate statistically significant difference between sealers (column), while keeping type of activation and root third unchanged ($P<0.05$). Distinct lowercase letters indicate statistically significant difference between root thirds (rows), while keeping sealer and type of activation unchanged ($P<0.05$). No statistically significant difference was detected between types of activation for any sealer and root third ($P<0.05$)

CONSIDERAÇÕES FINAIS

A resistência de união dos materiais empregados e a qualidade da obturação dos canais radiculares são pontos importantes para o sucesso da terapia endodôntica. A literatura demonstra que diversos fatores clínicos podem influenciar tais propriedades, como o tipo de cimento e a técnica obturadora (ØRSTAVIK, 2014), além de fatores metodológicos relacionados às pesquisas (BRICHKO; BURROW; PARASHOS, 2018; COLLARES et al., 2016; KIM et al., 2018).

Evidências clínicas e científicas suportam o uso de cimentos à base de resina epóxica, associados à guta-percha, para a obturação do sistema de canais radiculares (ØRSTAVIK, 2014). Contudo, os cimentos biocerâmicos vêm assumindo grande importância no cenário endodôntico, em função da sua notória biocompatibilidade e capacidade de induzir a formação de tecido mineralizado (JITARU et al., 2016).

Neste estudo, comparou-se a resistência de união de dois novos cimentos endodônticos, Sealer Plus (resinoso) e Sealer Plus BC (biocerâmico), com o "padrão-ouro" AH Plus (resinoso). Verificou-se que o cimento Sealer Plus apresentou maior resistência de união em relação ao cimento Sealer Plus BC, quando considerado o terço apical da raiz e os terços agrupados. Esse cimento também demonstrou valores de resistência de união maiores que o AH plus no segmento apical.

O uso do ultrassom tem sido proposto em diversos procedimentos endodônticos (PLOTINO et al, 2007), inclusive na ativação de cimentos endodônticos (WIESSE et al., 2018). O presente estudo constatou que entre os grupos onde foi realizada a ativação ultrassônica do cimento, não houve diferença significativa quanto à resistência de união. Porém, ao comparar os grupos com e sem agitação ultrassônica, o cimento biocerâmico Sealer Plus BC apresentou um aumento significativo na sua resistência de união no terço apical e nos terços agrupados. A resistência de união dos cimentos resinosos não foi influenciada pelo uso do ultrassom. Quando os segmentos da raiz foram comparados, os resultados não indicaram diferença significatina na resistência de união.

O modo de falha induzido após o teste *push-out* também foi avaliado neste estudo, sendo que falha mista prevaleceu em todos os grupos experimentais. Houve

100% de falha mista no terço médio para o cimento AH Plus e no terço apical para o cimento Sealer Plus.

A qualidade da obturação pode ser julgada pela presença de espaços vazios, os quais são encontrados com grande frequência na massa obturadora (GANDOLFI et al., 2012; KIM et al., 2018). Por meio da análise da obturação em estereomicroscópio, pode-se observar que o cimento biocerâmico Sealer Plus BC apresentou uma pior qualidade de obturação em comparação ao Sealer Plus, no terço apical da raiz e nos terços agrupados. Entre os cimentos ativados por ultrassom, não houve diferença significativa. A ativação ultrassônica não reduziu a presença de espaços vazios em nenhum dos cimentos avaliados. Houve uma tendência de mais espaços vazios no terço apical, sendo a diferença significante para os cimentos AH Plus, com ou sem ativação ultrassônica, e Sealer Plus BC, sem ativação.

Os resultados aqui apresentados reforçam o corpo de evidências científicas sobre os cimentos endodônticos resinosos e biocerâmicos. Contudo, novos estudos são necessários para analisar o comportamento dos novos cimentos, a fim de consolidar sua utilização na prática clínica.

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ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF ENDODONTICS*

american association of endodontists

Guidelines for Publishing Papers in the JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and reviews articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students, and scientists. Effective communication with this diverse readership requires careful attention to writing style.

General Points on Composition

1. Authors are strongly encouraged to analyze their final draft with both software (e.g., 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 since 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 have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the *JOE*:

2. 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.
3. 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.
4. Sentences are stronger when written in the active voice, *i.e.*, 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.
5. 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.)

6. 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.
7. 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."
8. 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.
9. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure 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 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.

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Organization of Original Research Manuscripts

Please Note: *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e., *Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

1. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, 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 icmje.org). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and order reprints when applicable. The contribution of each author should also be highlighted in the cover letter.
2. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings, and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.
3. **Introduction:** The introduction should briefly review 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. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing

the extensive review or the literature, and discuss the results of the study in this section.

4. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components of this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either 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, the authors must describe experiments made to validate the **model**, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.
5. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). 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 should be described in their order of numbering with a brief description of the major

findings. The author may consider the use of supplemental figures, tables or video clips that will be published online. Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).

6. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photos, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multi-panel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for the maximum number of allowable figures).The second type of figures is graphs (*i.e.*, 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). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for 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.
7. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However,

not all results need be placed in either a table or figure. For example, the following table may not be necessary:Instead, the results could simply 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). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).

% NaOCl	N/Group	% Inhibition of Growth
0.001	5	0
0.003	5	0
0.01	5	0
0.03	5	0
0.1	5	100
0.3	5	100
1	5	100
3	5	100

8. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical leadership. Ideally, a review of the potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions
9. **Acknowledgments:** All authors must affirm that they have no financial affiliation (e.g., 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 three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise, the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed

10. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the JOE. The JOE uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed 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 to the number of references for review articles.

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Manuscripts Category Classifications and Requirements

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

1. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at consort-statement.org. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
2. Review Article-Manuscripts in this category is either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by the extensive review of the literature will be categorized as "Case Report/Clinical Technique". These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit

of a total of 4 figures and 4 tables*.

3. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
4. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.
5. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.
6. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion, and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables** Figures, if submitted as multi-panel figures must not exceed 1-page length. Manuscripts submitted with more than the allowed number of figures or tables will require the approval of the JOE Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at jendodontics@uthscsa.edu.Importantly, adhering to the general writing methods described

in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

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Available Resources

Strunk W, White EB. The Elements of Style. Allyn & Bacon, 4th ed, 2000, ISBN 020530902X.

Day R. How to Write and Publish a Scientific Paper. Oryx Press, 5th ed. 1998. ISBN 1-57356-164-9.

Woods G. English Grammar for Dummies. Hungry Minds:NY, 2001 (an entertaining review of grammar).

Alley M. The Craft of Scientific Writing. Springer, 3rd edition 1996 SBN 0-387-94766-3.

Alley M. The Craft of Editing. Springer, 2000 SBN 0-387-98964-1.

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ANEXO B – TERMO DE DOAÇÃO DE DENTES BOVINOS

UNIVERSIDADE FEDERAL DE SANTA MARIA
CURSO DE ODONTOLOGIA

TERMO DE DOAÇÃO DE DENTES BOVINOS

FRIGORÍFICO SILVA INDÚSTRIA E COMÉRCIO LTDA, situado na Estrada BR 392, Km 08, SN, Passo dos Tropas, Santa Maria/RS, sob CNPJ 88.728.027/0001-46 e Inscrição Estadual 1090096949, declara que forneceu 114 dentes bovinos necessários ao desenvolvimento da pesquisa intitulada "Efeito da ativação ultrassônica na resistência de união imediata e tardia de cimentos endodônticos resinosos e biocerâmicos" sob responsabilidade das pesquisadoras Prof. Dra. Renata Dornelles Morgental e Karine Padoin. Os dentes em questão são provenientes de animais abatidos com propósitos comerciais.

Santa Maria, 11 de dezembro de 2017.

Ariane Schüssler Franken

Ariane Franken
pervisora do Controle de Qualidade
Frigorífico Silva