Root filling quality and bond strength of endodontic sealers to human and bovine dentin

Qualidade da obturação e resistência de união de cimentos endodônticos à dentina humana e bovina

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Resumo

Objetivo: Este estudo teve como objetivo comparar a qualidade da obturação e a resistência de união de dois cimentos endodônticos, AH Plus e Bio-C Sealer, em dentes humanos e bovinos. Métodos: Os canais radiculares de 60 dentes unirradiculares [30 humanos (H) e 30 bovinos (B)] foram preparados e obturados por condensação lateral da guta-percha e AH Plus (grupos AP-H e AP-B) ou Bio-C Sealer (grupos BC-H e BC-B). Seis fatias de 1,5 mm de espessura foram obtidas de cada raiz. Os espécimes foram observados em estereomicroscópio para avaliar a qualidade da obturação, considerando possíveis espaços vazios no material obturador. Posteriormente, as fatias radiculares foram avaliadas em termos de resistência de união por *push-out* e modo de falha. Os dados foram analisados pelos testes de Mann-Whitney e coeficientes de correlação de Spearman (α=5%). Resultados: A qualidade de obturação fornecida por AP e BC foi semelhante em ambos os substratos de dentina. No entanto, ao comparar dentes humanos e bovinos, os escores de espaços vazios foram maiores nas amostras bovinas, para ambos os cimentos. AP teve maior resistência de união à dentina humana e bovina do que BC. No entanto, não houve diferença significativa na resistência de união entre os substratos dentinários, para ambos os cimentos testados. Além disso, houve uma correlação positiva e moderada entre os valores de resistência de união de dentes humanos e bovinos. O modo de falha misto foi o mais prevalente. Conclusão: AP e BC fornecem qualidade de obturação semelhante, mas o primeiro apresenta maiores valores de resistência de união à dentina humana e bovina. A utilização de dentes bovinos como substitutos de amostras humanas parece ser adequada em estudos relacionados à resistência de união, mas não naqueles que testam a qualidade da obturação endodôntica.

Palavras-chave: dentina; endodontia; materiais obturadores do canal radicular.

http://dx.doi.org/10.5335/rfo.v28i1.15084

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Introduction

The main goal of root canal filling is to promote a three-dimensional seal of the root canal system, after proper cleaning and shaping, to avoid the passage of microorganisms and their products to the periapical tissues[1]. In this context, the technical quality of the root canal filling impacts the success of endodontic treatment[2]. However, achieving an adequate obturation involves several challenges, such as difficulties related to the adhesion of root filling materials to the dentin walls[1-3].

Gutta-percha is the primary core material used in endodontics, but it cannot hermetically seal a root canal space since it has no adhesive qualities[3]. Thus, the use of an endodontic sealer is necessary for the union of the filling material to the dental structure, preventing the presence of gaps and voids[1]. This union will occur through frictional retention and micromechanical adhesion[4] and is influenced by several factors, such as dentin and gutta-percha surface energy, sealer surface tension, sealer flow and root canal cleaning[5]. Methodological variability may also impact the results of studies concerning the bond strength or dislocation resistance of endodontic materials[6].

AH Plus (AP) (Dentsply DeTrey GmbH, Konstanz, Germany), an epoxy resin-based sealer, is widely used in clinical practice and is available in a paste-paste form. Such material has been considered the ‘gold standard’ among endodontic sealers, due to its excellent physicochemical properties, including low solubility and long-term dimensional stability[7], as well as high bond strength to dentin[8,9].

In the last decade, a new class of calcium silicate-based endodontic sealers was developed, the so-called ‘bioceramic’ sealers. Such materials are available in a pre-mixed and ready-to-use form. They have received considerable attention from the scientific community because of their excellent biological properties and potential for biomineralization[9]. Bio-C Sealer (BC) (Angelus, Londrina, PR, Brazil) was launched in the market in 2018. Its physicochemical and biological behavior has been investigated with favorable results[10]. However, up to now, there is scarce information in the scientific literature about the bond strength of this material to the root canal walls[11].

The use of human teeth in laboratory research is hampered by ethical issues, as well as problems related to obtaining the appropriate sample size and specimen standardization. Thus, various dentin substrates have been suggested as substitutes for human teeth in *in vitro* studies, including bovine, equine and ovine teeth[12-14]. Bovine dentin has been widely used as an alternative to human dentin in bond strength studies, as they share similar macro and microscopic characteristics[13-14]. However, this dentin substrate also receives criticism, especially regarding the number of dentinal tubules in the root portion, which is higher than that of the human dentin substrate, although the diameter of the tubules is similar[15].

In this context, further investigation on the properties of endodontic sealers in bovine-tooth models is warranted, in comparison to human samples. Thus, this study aimed to compare the filling quality and bond strength of two endodontic sealers, AH Plus (resinous) and Bio-C Sealer (bioceramic), in human and bovine teeth. The following null hypotheses were established: 1) there is no difference in the filling quality provided by the two sealers; 2) there is no difference in the filling quality observed in human and bovine teeth; 3) there is no difference in the bond strength of the two sealers; 4) there is no difference in the bond strength to human and bovine teeth.

Materials and method

This study was approved by the local Research Ethics Committee (protocol number 92736618.0.0000.5346).

Sample selection

The sample size was determined using the online software OpenEpi version 3.01, based on the parameters of Galhano et al.[16] (2009): bond strength of 8.6 (± 5.7) MPa in human teeth and 4.1. (± 1,3) MPa in bovine teeth; 80% power; 5% significance level. A minimum number of 14 teeth per group was established. However, considering the variability of dental root anatomy, 15 teeth per group were used. Thus, 30 human and 30 bovine single-rooted teeth were selected for this study.

The teeth were previously observed in a stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany), with 8× magnification, to exclude those with cracks or fractures, incompletely formed apex and root resorption. Organic remains on the root surface were removed by periodontal curettes (Golgran, São Paulo, SP, Brazil). Tooth disinfection was performed by immersion in a 0.5% T-chloramine solution (Sigma-Aldrich Brazil LTDA, Duque de Caxias, RJ, Brazil) for one week and then in distilled water to remove residues from the product.

The coronal portion of the teeth was sectioned with carborundum discs (KG Sorensen, Barueri, SP, Brazil) to obtain root remnants with a standardized length of 16 mm. For human teeth, the anatomical diameter of the root canal should correspond to a size 35 K-file (Dentsply-Maillefer, Ballaigues, Switzerland); while for bovine teeth, roots with an anatomical diameter of the canal corresponding to a size 60 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) were selected, excluding excessively large canals. The specimens were stored in distilled water at 4ºC until the moment of the experiments.

Root canal preparation

In human teeth, the canals were initially irrigated with 5 ml of 2.5% sodium hypochlorite (NaOCl) solution (Biodynamic Chemical and Pharmaceutical LTDA, 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.

During the chemomechanical preparation, the crown-down technique was employed, using Gates-Glidden drills and hand stainless steel instruments (Dentsply-Maillefer, Ballaigues, Switzerland). The cervical and middle thirds of the canals were prepared with size 3 and 2 Gates-

Glidden drills, respectively. The apical third was instrumented with K-type files, from a size 35 to a size 50 K-file.

All procedures were performed under abundant irrigation with 2.5% NaOCl, using 2 ml of irrigating solution at each instrument change. The irrigation process was performed with a 5 ml plastic syringe and Navi Tip 31ga / 27 mm tip (Ultradent Products Inc., South Jordan, UT, USA). Final irrigation was performed with 5 ml of 17% EDTA solution (Chemical and Pharmaceutical Biodynamics LTDA, Ibiporã, PR, Brazil) for 5 minutes to remove the smear layer and then the specimens were irrigated with 10 ml of distilled water[8].

For chemomechanical preparation in bovine teeth, irrigation, WL determination, and instrumentation were performed similarly to that described above. The cervical and middle thirds were prepared with size 5 and 4 Gates-Glidden drills (Dentsply-Maillefer, Ballaigues, Switzerland), in this order, and the apical third was prepared with K-type files, starting at a size 60 until reaching a size 90 K-file (Dentsply-Maillefer, Ballaigues, Switzerland).

Randomization and distribution of experimental groups

The teeth were randomly distributed into two experimental groups (n=15/group) for each dentin substrate, using www.randomization.com, according to the endodontic sealer used in the root filling (Table 1): AP-H Group: AH Plus in human teeth; AP-B Group: AH Plus in bovine teeth; BC-H Group: Bio-C Sealer in human teeth; BC-B Group: Bio-C Sealer n bovine teeth.

Table 1 – Composition of endodontic sealers and their manufacturers.

Sealer	Composition	Manufacture
AH Plus	Paste A: bisphenol-A epoxy resin; bisphenol-F epoxy resin; calcium tungstate; zirconia oxide; silica and iron oxide. Paste B: adamantine amine; n, n'' -dibenzyl-5 oxanoname diamine-1,9; TCD-diamine; calcium tungstate; zirconia oxide; silica and silicone oil.	Dentsply, DeTrey GmbH, Konstanz, Germany
Bio-C Sealer	Calcium silicates, calcium aluminate, calcium oxide, zirconia oxide, iron oxide, silicon dioxide and dispersing agent.	Angelus, Londrina, PR, Brazil

After chemomechanical preparation, root canals were dried with absorbent paper points (Dentsply Brasil, Petrópolis, RJ, Brazil). Size 50 and 90 gutta-percha points (Dentsply Brasil, Petrópolis, RJ, Brazil) for human and bovine teeth, respectively, were tested for tug-back at the WL and the apical position was radiographically confirmed.

Next, the root canals were filled with the respective endodontic sealer, which was manipulated according to the manufacturer's instructions. The sealer was carried into the canal with a size 4 Lentulo spiral (Dentsply-Maillefer, Ballaigues, Switzerland) at low speed for 5 seconds. This procedure was repeated (up to three times) until the root canal walls were completely cover by the sealer. The main gutta-percha point was inserted into the full WL and obturation was complemented by lateral condensation technique using finger spreaders (Dentsply-Maillefer, Ballaigues, Switzerland) and accessory gutta-percha points (Dentsply-Maillefer, Ballaigues, Switzerland). After radiographic confirmation of complete root canal filling, excess material was removed with a heated instrument (Golgran, São Paulo, SP, Brazil), followed by vertical condensation. The cervical portion of the roots was sealed with a temporary restorative material (Coltosol; Coltene, Altstätten, Switzerland) and stored at 37ºC and 100% humidity for 24 hours to allow the sealers to set.

Specimen preparation

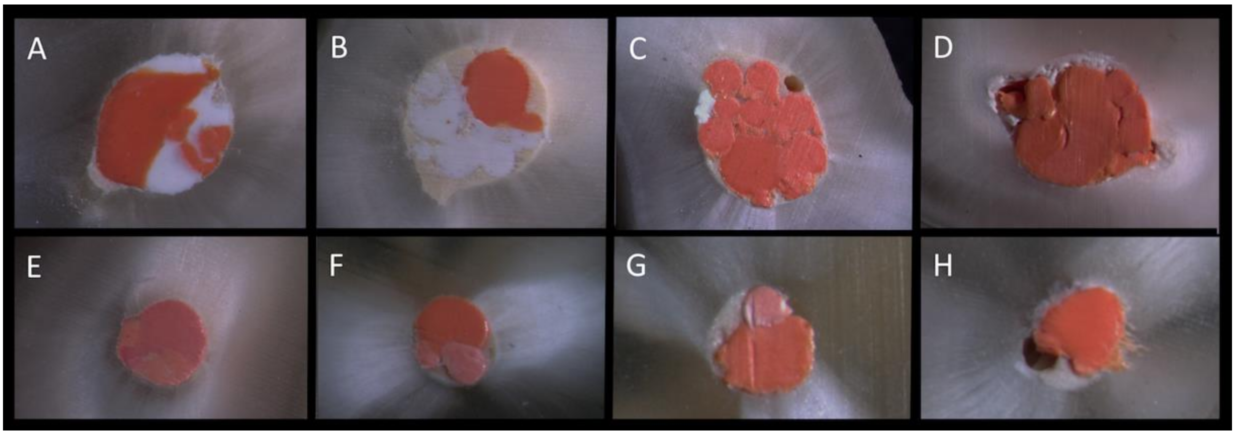
The roots were transversely sectioned on a precision cutting machine (LabCut 1010; Extec Inc., Enfield, CT, USA) set at 300 rpm and equipped with a double-sided diamond disc (Buehler, Lake Bluff, IL, USA). To facilitate their fixation in the machine, the cervical portion of the roots (about 2 mm) was included in self-cured acrylic resin (Clássico Artigos Odontológicos, Campo Lindo Paulista, SP, Brazil). The most cervical and apical portions of each root were discarded, and six slices of 1.5 (± 0.3) mm thickness were produced.

Filling quality assessment

All slices were observed under a digital stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany) at 25× and 40× magnification for filling quality analysis. The digital images obtained were evaluated to estimate the presence, number, and diameter of voids within the filling material, using the scoring system proposed by Kim et al.[17] (2018). Void diameters were calculated by the ImageJ 1.46 software (National Institutes of Health, Bethesda, MD, USA) was used with a standardized 75% zoom.

The filling quality was evaluated by a single calibrated examiner (weighted kappa = 0.77), who was blinded to the experimental groups. The four scores were as follows[17]: 1) well-condensed filling with only a few, minor air bubbles (<0.1 mm in diameter); 2) an imperfectly condensed filling with some minor air bubbles (more than 3 defects) or medium-sized air bubbles (0.1 to 0.2 mm in diameter); 3) inadequately condensed filling with many minor air bubbles (more than 5 defects) or large air bubbles (> 0.2 mm in diameter); 4) poorly condensed filling, having many minor air bubbles (more than 7 defects) or empty space connecting two or more root canal walls (Figure 1).

Figure 1 – Representative images of filling quality scores obtained by stereomicroscope. (A-D) Bovine teeth: (A) Score 1; (B) Score 2; (C) Score 3; (D) Score 4. (E-H) Human teeth: (E) Score 1; (F) Score 2; (G) Score 3; (H) Score 4.



Bond strength evaluation

The push-out test was performed in a universal testing machine (EMIC DL-2000; EMIC, Sao Jose dos Pinhais, PR, Brazil) by a single trained operator. The root slices were positioned in the machine with their cervical surfaces facing down on a metal device with a 4-mm-diameter opening. The root canal orifice was centered on this opening. Next, a compressive load was applied with a metal cylinder (0.4, 0.6 or 0.8-mm-diameter tip, depending on the root third), touching the center of the filling material. The load was introduced in apical-cervical direction (1 mm/min speed), and the bond strength (σ) was obtained in megapascal (MPa), as described in a previous study[18]. The following formula was applied: $\sigma = F/A$, where F = load for filling dislodgement (N) and A = adhesion area (mm^2). For A determination, 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, and 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 bases, respectively, corresponding to the internal diameters of the root canal walls. H, R_1, and R_2 were measured after the test with a digital caliper (Mitutoyo, Suzano, SP, Brazil), by the same operator.

Failure mode analysis

For analysis of the failure mode induced during the push-out bond strength test, root slices were again examined under a digital stereomicroscope (Stereo Discovery V20; Zeiss, Oberkochen, Germany) at 25× and 40× magnification by a blinded and calibrated examiner (kappa = 0.70). The failure patterns were classified as: adhesive, when the sealer was entirely separated from the dentin (surface without sealer); cohesive, when the failure occurred within the filling material (surface entirely covered by sealer); and mixed, when both adhesive and cohesive failure modes were verified (surface partially covered by sealer)[19].

Statistical analysis

Data were first analyzed for normality by the Shapiro-Wilk test. Because there was no normal distribution, nonparametric tests were employed. Comparisons between endodontic sealers and dentin substrates were performed by Mann-Whitney tests for filling quality and bond strength. The results of human and bovine teeth were also analyzed by Spearman correlation coefficients. All analyses were performed using the SPSS Statistics software (version 20; SPSS Inc., Chicago, IL, USA), considering a 5% significance level.

Resultados

Regarding the filling quality, AP and BC showed similar scores (P <0.05) in both human and bovine teeth. When comparing the two dentin substrates, filling quality was worse (higher void score) in bovine teeth than in human ones (P <0.05) for both sealers tested (Table 2). There was no significant correlation between the results of human and bovine samples (r = -0.08; P = 0.258).

Table 2 – Void scores according to endodontic sealer and dentin substrate.

Sealer	Dentin substrate										
	Human						Bovine				
	N	Scores				Median	Scores				Median
		1	2	3	4		1	2	3	4	
AP	90	30	25	29	6	2^B	23	13	24	30	3^A
BC	90	27	23	29	11	2^B	8	14	37	31	3^A

AP: AH Plus; BC: Bio-C Sealer.
Distinct letters indicate significant difference between dentin substrates (in each row).
There was no significant difference between the sealers (in each column).

Regarding the bond strength, as shown in Table 3, AP presented significantly higher values than BC (P <0.05) in both human and bovine teeth. However, no significant difference was detected when comparing the bond strength of the two dentin substrates (P> 0.05) for either sealer. In addition, there was a positive and moderate correlation between the results of human and bovine teeth (r = 0.44; P = 0.015).

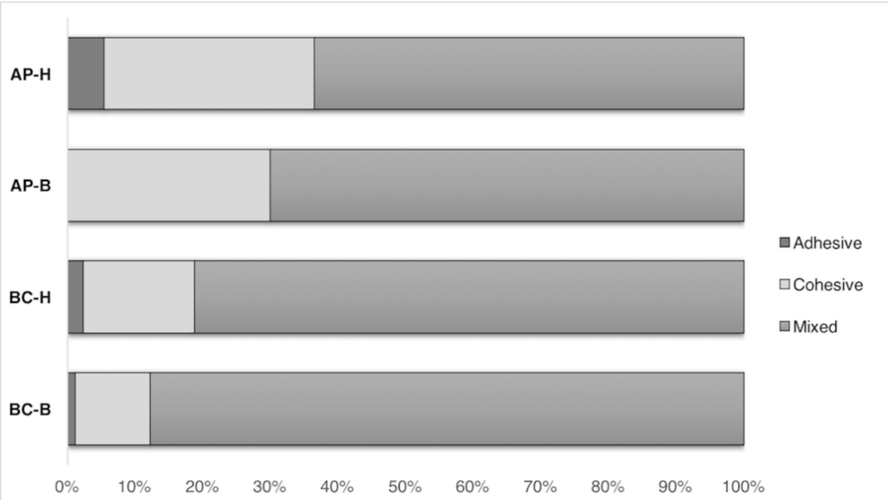
Table 3 – Bond strength (mean ± standard deviation; MPa) according to endodontic sealer and dentin substrate.

Sealer	Dentin substrate		P
	Human	Bovine	
AP	4,16 ± 2,22[A]	3,54 ± 1,60[A]	0,386
BC	1,81 ± 0,86[B]	1,48 ± 1,31[B]	0,416
P	0,00069	0,00065	

AP: AH Plus; BC: Bio-C Sealer.
Distinct letters indicate significant difference between the sealers (in each column).
There was no significant difference between dentin substrates (in each row).

The mixed failure mode was the most prevalent in all experimental groups (Figure 2). Adhesive failure was observed in very few specimens, and cohesive failure was more prevalent in AP than in BC in both dentin substrates.

Figure 2 – Failure mode distribution (%) according to endodontic sealer and dentin substrate. AP: AH Plus; BC: Bio-C Sealer; H: human; B: bovine.



Discussion

New dental materials are continually launched into the market and can be used in clinical practice. However, the choice of appropriated materials should be guided by laboratory and clinical studies, showing the real advantages and disadvantages of these new products. To the best of our knowledge, this in vitro study was the first to evaluate the filling quality and bond strength of the new BC endodontic sealer, compared to the 'gold standard' AP, considering human and bovine teeth as substrate. Two of the four proposed null hypotheses were rejected since the filling quality was worse in bovine teeth than in human ones, and AP presented higher bond strength than BC.

Regarding the filling quality, AP and BC showed similar behavior when tested in both human and bovine samples. Previous studies using other bioceramic sealers compared to AP showed the same results regarding the presence of voids within the root filling[20,21]. One could assume that the flow property of the sealer influences root canal filling quality. The higher the flowability of the material, the greater its ability to penetrate the root canal system, with lower chance of voids and gaps[1]. According to Zordan-Brondel et al.[22] (2019), BC has adequate flow (> 20 mm), even higher than AP, following the recommendations of ISO 6876:2012[23]. This finding may explain the fair obturation capacity of both bioceramic and resinous sealers, agreeing with the results of this study.

Calcium silicate-based endodontic sealers have been extensively studied for their bond strength to root canal walls[9,24,25]. Some researches show a similarity between bioceramic sealers and AP[24,25], while others demonstrate the superiority of the epoxy resin-based sealer[26], as found in the present study. The excellent adhesive property of epoxy resin-based materials can be explained by the formation of covalent bonds with any amine group exposed in the dentin collagen when its epoxide ring opens[27], in addition to its good dimensional stability[7].

The lower bond strength of the bioceramic sealer may have been a consequence of an inadequate hydration process during the experiments, as suggested by Carvalho et al.[26] (2017). The setting of premixed calcium silicate-based sealers depends on the presence of moisture in the dentinal tubules, thus an excessive drying of root canals is contraindicated. Nagas et al.[28] (2012) observed that in order to favor the bond strength of iRoot SP bioceramic sealer, the canal must be partially moist, i.e., dried with only one absorbent paper point. Although such maneuver was performed in the present study, it is difficult to predict if dentin moisture was actually preserved. Thus, a possible insufficient presence of water may have led to an imperfect or incomplete setting process.

The failure mode induced by the push-out test was also evaluated in this study, and the mixed failure prevailed in all experimental groups. These findings are in agreement with previous studies[8,19]. However, there is no consensus in the endodontic literature regarding the failure pattern observed with resinous and bioceramic sealers[27,28].

Obtaining sufficient extracted human teeth in good condition for laboratory research has become difficult due to the decrease in the prevalence of caries in recent decades and the use of more conservative restorative approaches[13]. Such a scenario has led to the use of alternative substrates. The use of bovine dentin has advantages related to specimen standardization since several bovine teeth can be obtained from fewer animals, minimizing confounding factors such as tooth age, occlusal condition, and diet[16].

According to the systematic review and meta-analysis conducted by Soares et al.[13] (2016), the bond strength values of adhesive systems are similar in human and bovine teeth for both enamel and dentin substrates. Thus, bovine teeth could be considered as suitable substitutes for human teeth in bond strength tests. However, most of the studies included in the analysis evaluated the bond strength of adhesive materials to coronal dentin, and only one investigation assessed the adhesion of glass fiber posts to root dentin[16]. Regarding endodontic sealers, the systematic review and meta-analysis by Collares et al.[12] (2016) showed that bovine root dentin presented higher bond strength than human dentin. However, this analysis considered only two studies with bovine teeth and 37 with human teeth, without direct comparison between the two dentin substrates, with high methodological variation among the researches included in the analysis.

In the present study, the bond strength to human and bovine dentin was similar for both sealers tested (AP and BC), indicating that bovine teeth can be used in laboratory tests without great divergence of results. In addition, there was a positive and moderate correlation between the results of human and bovine teeth. The study by Silva et al.[29] (2019) also demonstrated that the bond strength of endodontic sealers is similar in human and bovine dentin. However, these authors used an experimental model where three standardized artificial holes were made in the same root slice (around the canal), filled with different sealers and submitted to the push-out test. This model has the advantage of hole standardization but does not fully reproduce the procedures performed in clinical practice.

When comparing void scores in the obturation mass, the values were higher for bovine than human teeth, characterizing a worse filling quality. This finding was already expected and can be explained by the larger diameter of the root canals in the bovine teeth, combined with the inability of the cold lateral condensation technique to promote a completely dense and homogeneous root filling, particularly in large canals[30,31]. Moreover, no significant correlation was detected between the results of human and bovine teeth, suggesting that the latter would not be proper substitutes for the evaluation of filling quality.

Although carefully delineated, this laboratory study represents only an estimate of the filling quality and immediate bond strength promoted by the new BC sealer compared to the “gold standard” AP. These findings should be complemented by long-term in vitro studies and further confirmed by randomized controlled trials to understand the actual clinical behavior of this new material.

Conclusion

It can be concluded that AP and BC provide similar filling quality, but the former has higher bond strength to human and bovine dentin. The use of bovine teeth as substitutes for human ones seems appropriate in studies related to the bond strength of endodontic sealers since the results on both dentin substrates were similar and positively correlated. However, bovine teeth are not recommended in studies on filling quality, since the results in human and bovine samples were different and not correlated.

Abstract

Objective: This study aimed to compare the filling quality and bond strength of two endodontic sealers, AH Plus and Bio-C Sealer, in human and bovine teeth. Methods: The root canals of 60 [30 human (H) and 30 bovine (B)] single-rooted teeth were prepared and filled by lateral condensation of gutta-percha and AH Plus (groups AP-H and AP-B) or Bio-C Sealer (groups BC-H and BC-B). Six 1.5-mm-thick slices were obtained from each root. The specimens were observed under a stereomicroscope to assess filling quality, considering possible voids within the filling material. Subsequently, root slices were evaluated in terms of push-out bond strength and failure mode. Data were analyzed *by Mann-Whitney tests and Spearman correlation coefficients (α=5%). Results: The filling quality provided by AP and BC was similar in both dentin substrates. However, when comparing human and bovine teeth, void scores were greater in the bovine samples, for both sealers. AP had higher bond strength to human and bovine dentin than BC. However, there was no significant difference in bond strength between dentin substrates, for both sealers tested. Also, there was a positive and moderate correlation between the bond strength values of human and bovine teeth. The mixed failure mode was the most prevalent. Conclusion: AP and BC provide similar filling quality, but the first presents higher bond strength values to human and bovine dentin. The use of bovine teeth as substitutes for human samples seems adequate in studies related to bond strength, but not in those testing root canal filling quality.*

Keywords: dentin; endodontics; root canal filling materials.

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Recebido em: 01/08/2023. Aceito: 01/09/2023.