



Filling Material Bond Strength to Dentin Is Positively Influenced by the Agitation of Endodontic Final Irrigating Solutions

Alessandra Aparecida da Silva Trandafilov^a , Renata Grazziotin-Soares^b , Amanda Palmeira Arruda Nogueira^a , Etevaldo Matos Maia-Filho^a , Meire Coelho Ferreira^a , Rudys Rodolfo de Jesus Tavaréz^a , José Bauer^c , Ceci Nunes Carvalho^{a*}

^a School of Dentistry, Ceuma University, São Luís, Maranhão, Brazil; ^b Faculty of Dentistry, The University of British Columbia (UBC), Vancouver, Canada; ^c Discipline of Dental Materials, School of Dentistry, University Federal of Maranhão (UFMA), São Luis, MA, Brazil

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*Corresponding author: Ceci Nunes Carvalho, School of Dentistry, CEUMA University, Rua Josué Montello, Renascença II, 65075120, São Luís, MA, Brazil.

E-mail: ceci.carvalho@ceuma.br

Introduction: The final step of irrigation has been considered to increase the bonding strength of filling material to dentin. This study investigated the impact of three final-step irrigation methods on the endodontic sealer bond strength to dentin by using a micro push-out test. **Materials and Methods:** Palatal roots of human maxillary molars were cleaned and shaped and randomly divided in six groups (n=15) according to the final-step irrigation method and the type of root canal sealer used. The solutions used for the final-step irrigation were 17% ethylenediaminetetraacetic acid and 2.5% sodium hypochlorite, which underwent three methods: 1) syringe-needle irrigation/conventional, 2) passive ultrasonic irrigation, and 3) XP-endo Finisher agitation. The root canal sealers used were: EndoSequence BC Sealer, and AH-Plus sealer. Roots were obturated with the single cone technique and then, cross-sectioned in 2-mm-thick slices (3 slices from each root). Push-out test was performed on the sliced specimens (cervical, middle, and apical thirds) with a universal testing machine. Bond strength values were recorded in megapascal (MPa). Subsequently, each specimen was longitudinally split to verify the type of failure. Data analysis was performed using Johnson transformation, three-way analysis of variance, Tukey's post-hoc tests, and the partial Eta squared test. **Results:** There were significant differences in bond strength between the sealers [AH: 4.46 ± 2.24 and BC: 3.47 ± 2.19 MPa ($P < 0.001$)]; between final-step irrigation methods [passive ultrasonic irrigation: 4.52 ± 2.25 , XP-endo Finisher: 3.93 ± 3.93 and syringe-needle irrigation/conventional: 3.37 ± 2.51 MPa ($P < 0.001$)], and between the root canal thirds represented by the sliced specimens [cervical: 5.45 ± 2.39 , middle: 4.14 ± 1.99 and apical: 2.30 ± 1.30 MPa ($P < 0.001$)]. The interaction between the variables had no significance ($P > 0.05$). **Conclusion:** Agitation of the final irrigating solution may improve the bonding of the sealer to canal walls. AH-Plus sealer had the highest bond strength. The bond strength reduced significantly towards the apical third.

Keywords: Bond Strength; Final Flush; Final-Step Irrigation Methods; Outshout Test; Passive Ultrasonic Irrigation; Root Canal Treatment; XP-endo Finisher

Introduction

The bond strength, or the dislodgment resistance, of endodontic fillings in relation to the canal walls is fundamental in endodontic therapy because it guarantees appropriate adaptation and integrity between the interfaces [1-4]. Both adaptation and integrity ultimately improve biological results for the patient i.e., less (or none) percolation of fluids and

microorganisms to the already cleaned and obturated root canal [5]. Endodontists expect high bond strength between the filling material (gutta-percha + sealer) and the root canal dentin walls, and this is derived from micromechanical retention (frictional resistance) and adhesion between the interfaces [6-8].

The final step of irrigation, also known as the final flush of irrigation, performed immediately before the root canal obturation, has been considered to act in favor of endodontic

treatment success, because it can augment the bonding of endodontic filling material to the canal walls. After cleaning and shaping, a smear layer composed of bacteria and microorganisms, necrotic debris, precipitates originated from the interaction of irrigants, and dentin debris is formed on the canal walls [9-11]. The removal of this layer is crucial for acquiring an optimal interaction between filling material and dentin [12-14].

Several main irrigation techniques were studied in relation to the influence on the filling material bond strength. Evidence shows that irrigation with conventional syringe needle is ineffective in removing dentinal debris and cleaning the apical third of the root canal [15, 16]. Therefore, alternative cleaning techniques have been investigated. Agitation methods, such as photon-initiated photoacoustic streaming, and ultrasonic and sonic agitation of irrigation solutions had positive effects on the bond strength of filling materials (different sealers, and fiber-posts) to dentin [17, 18]. Similarly, the XP-endo Finisher file, when used to agitate the irrigation solution has facilitated the removal of intracanal medication (calcium hydroxide) and improved the epoxy-resin sealer bond strength to dentin, when compared to other irrigation protocols, including passive ultrasonic irrigation (PUI) and syringe-needle conventional irrigation (IC) [19]. Additionally, the XP-endo Finisher R instrument removed significantly more root filling material than PUI [20, 21].

Particularly regarding the final step of irrigation, authors reported that the sealer bond strength to dentin depends on the combination of different irrigants and the technique of flushing; in other words, it depends on the interaction between the variables [22, 23]. For instance, the use of chlorhexidine after sodium hypochlorite as a final irrigation protocol increased the bond strength of AH-Plus (epoxy resin-based) sealer (DeTrey/Dentsply, Ballaigues, Switzerland), but did not create any difference in bond strength to dentin for calcium silicate-based sealers [24]. Similar to studies that investigated main irrigation techniques, the studies investigating final-step irrigation showed that the agitation of the solution, or the combination of solutions, may improve filling material's bond strength to dentin [25-27].

Apart from the final-step irrigation method, the type of sealer and the root canal locations have influence on filling material bond strength to dentin. AH-Plus is considered a gold standard sealer regarding the bond strength in *in vitro* evaluations, because of its history of clinical success. Several studies found higher bonding strength for AH-Plus, when compared to other sealers [26-31]. The apical third of the canal is where the lowest bond strength in the interface of sealer-dentin is found because of its anatomical challenges imposed during cleaning and shaping [32-34].

Considering previous evidence, the tested hypotheses for the present study were that the final-step irrigation method would influence the sealer bond strength to dentin, assuming that the solution agitation with XP-endo Finisher would promote higher bond strength than PUI. In addition, we assumed higher bond strength for when using AH-Plus, and in the cervical third of the root canal. Therefore, this study investigated the influence of three final-step irrigation methods (IC; PUI; and XP-endo Finisher agitation) on the root canal sealer bond strength to dentin using a micro push-out test.

Material and Methods

In this *in vitro* study, we used human teeth to measure the bond strength of root canal sealers to dentin walls depending on the final-step irrigation method and the type of root canal obturation sealer (independent variables). The project protocol was approved by the university ethical board (#1.939.590/2016). Two root canal sealers were used: 1) EndoSequence BC Sealer (BC; Brasseler, Savannah, GA, EUA), and 2) AH-Plus sealer (AH; Dentsply Maillefer Ltda., Petropolis, RJ, Brazil). The solutions used for the final-step irrigation were 17% ethylenediaminetetraacetic acid (EDTA) and 2.5% sodium hypochlorite (NaOCl) that were utilized in three different methods of irrigation: 1) IC, 2) PUI, and 3) XP-endo Finisher agitation. Ethical approval for the trial protocol was obtained from the CEUMA University Research Ethics Committee (#1.939.590) and teeth were obtained through donation.

Teeth preparation

Ninety unidentifiable and untraceable extracted maxillary first molars were collected. The exclusion criteria based on visual inspection under magnification, and radiographic inspection were root canal calcification, resorption, or root canal filling material. Crowns, at the level of cemento-enamel junction (CEJ), and buccal roots were separated by using a precision cutting machine (Isomet 100 Precision Saw; Buehler, Lake Bluff, IL, USA). Palatal roots were stored in 0.1% aqueous thymol for one week to be disinfected, and were later stored in distilled water at 4°C until use.

Root canal working length for each tooth was determined at 1 mm short of the apical foramen and was visualized under magnification after inserting a size #10 K-file. Root canal instrumentation was performed with a new R40 reciprocating instrument (Reciproc, VDW, Munich, Germany), under irrigation with 10mL of 2.5% NaOCl with a pH of 11. Root canals were dried with #R40 absorbent paper points for 3 seconds. The root apex was covered with cyanoacrylate gel bond (Super

Bonder, 3M, Itapevi, SP, Brazil). Root canal instrumentation, final-step irrigation, and root canal obturation were performed on the same day.

Roots were randomly divided in six groups ($n=15$) according to the final-step irrigation method and the type of root canal sealer used for obturation. Teeth were categorized in small containers and coded with numbers from 1-90. A master list was created and digitally stored, linking the numerical code with the two variables, final irrigation method and sealer.

Final-step irrigation

Three different methods of final-step irrigation were the following:

- 1) Syringe-needle irrigation/conventional: 5 mL of heated 17%-EDTA was inserted in the root canal with a 30-gauge needle (Max-i-Probe; Dentsply Maillefer, Ballaigues, Switzerland) for 30 sec. Ethylenediaminetetraacetic acid was heated using a heating plate and a Becker container [35]. Subsequently, the canal was irrigated with 5 mL of 2.5% NaOCl, and a final flush with 10 mL of deionized water by a 30-gauge irrigation needle (Max-i-Probe; Dentsply Maillefer). A #R40 absorbent paper point was used for 3 sec to dry the canal [36].
- 2) Passive ultrasonic irrigation: 1 mL of heated 17%-EDTA was flushed. An endodontic tip [15/0.02 (Helse, Santa Rosa de Viterbo, SP, Brazil)] attached to an ultrasonic device (EMS, Nyon, Sweden) was inserted up to 2 mm of the working length without touching the canal walls to allow the tip to vibrate freely at a power of 25%. Ethylenediaminetetraacetic acid was re-injected at each 30-second interval, for a total volume of 5 mL. Subsequently, 5 mL of 2.5% NaOCl was vibrated identical to the technique used for EDTA [37]. Final flush was performed with 10 mL of deionized water.
- 3) XP-endo Finisher agitation: 1 mL of heated 17%-EDTA was flushed into the root canal. An XP-endo Finisher endodontic instrument (size 25/0.00) attached to a slow-speed hand piece (VDW, Munich, Germany), cooled with endo-ice spray (Endo-Frost, Roeko, Langenau, Germany), was removed from its plastic tube, and inserted into the root canal without rotation until reaching the working length. Rotation was then activated at a speed of 800 rpm and a torque of 1 N.cm, and the instrument was vertically motioned with up and down movements for 30 seconds. Ethylenediaminetetraacetic acid was re-injected at each 30-sec interval, for a total volume of 5 mL. Subsequently, 5 mL of NaOCl was agitated identical to the technique used for EDTA [38]. Final flush was done with 10 mL of deionized water.

Root canal obturation

Both sealers underwent the single-cone obturation technique. Manipulation and handling were done according to the manufacturer recommendations. After drying the root canal, a

single #R40 gutta-percha cone (Reciproc, system VDW, Munich, Germany) was inserted into the canal gently, covered with sealer. The gutta-percha cone was cut off 2 mm below the canal entrance by using a heated instrument. Filling material was vertically compacted with a cold instrument. The 2 mm unfilled coronal portion of the root canal was cleaned with a cotton pellet moistened with alcohol. Roots were restored with Cavit-W (3M ESPE, Seefeld, Germany). And were then stored in an incubator for 30 days at a 37°C, 100% humidity.

Producing sliced specimens and evaluating the bond strength

The bond strength of root canal sealers to dentinal walls was measured by utilizing a push-out test. For this, the roots were cross-sectioned in 2-mm-thickness slices (5 slices from each root). Slices were made by using the cutting machine with the aid of a 0.4 mm thick diamond disk, under constant refrigeration. The apical and cervical slices of the root canal of each filled root were discarded therefore, 3 slices remained from each root (representing the three root canal thirds: apical, middle, and cervical). Slices' thicknesses were measured with a digital caliper with 0.01 mm-precision (Mitutoyo MTI Corporation, Tokyo, Japan), and specimens were sorted in an ascending order, from apical to cervical. With the aim of measuring the bottom (apical) and the upper (cervical) diameter of each slice, pictures of each side of the slices were captured with a digital camera (Q-Color 5, Olympus, Tokyo, Japan) connected to a stereomicroscope (SZ61, Olympus America Inc., PA, USA) under 40× magnification. Then, pictures were transferred to Image J (National Institute of Health, Maryland, USA; <http://rsb.info.nih.gov/ij/>) to be measured.

Push-out test was performed as follows: the upper (cervical) surface of the specimen remained facing the base of the test machine (EMIC, Instron Ltda., São José dos Pinhais, PR, Brazil); therefore, the bottom (apical) surface of the specimen received the load of the machine's cylindrical plunger. Plunger diameter was selected individually for each specimen, being approximately 2 mm smaller in diameter than the specimen's diameter. This would prevent the plunger from touching the dentin around the filling material. The descending movement of the plunger occurred at the speed of 0.5 mm per min, until the extraction of the filling material from inside the specimen occurred. This was confirmed by the abrupt reduction of the load value. Using the load value reported in kilogram-force, it was possible to calculate the bond strength according to the formula: Bonding Strength = force/area, transforming the load at the time of extrusion (N) and dividing it by the adhesive area (mm²). The adhesive area was calculated by the formula of the truncated cone area:

$$A = \pi(R + r)\sqrt{h^2 + (R - r)^2}$$

Abbreviations and symbols correspond to parameters as follows:

A=the specimen surface area,

π =3.1416,

R=radius of the bigger cone,

r=radius of the smaller cone,

h=slice thickness.

Failure mode analysis

Immediately following the push-out test, each individual specimen was assessed to verify the mode of failure. For this, the specimens were split longitudinally in the buccolingual direction using a chisel and a hammer. One segment of each specimen was observed under 40× magnification with a stereo-microscope, to visually assess the amount of remaining filling material in relation to the amount of dentin, in percentage. The failure was categorized in adhesive, cohesive or mixed. Adhesive failure for when the segment had more than 75% of dentin without filling material. Cohesive failure for when the segment had less than 25% of dentin without filling material. Mixed failure for when the segment had between 25-75% of dentin without the filling material [6] (Figure 1).

Data analysis

Push-out test values in megapascal (MPa), evaluated by Shapiro-Wilk test, did not have a normal distribution ($P<0.05$). Data were then transformed using the Johnson transformation (Minitab Inc., State College, PA, USA). Push-out test values underwent three-way analysis of variance (ANOVA) and Tukey's post-hoc tests to detect a significant statistical difference between final-step irrigation methods, between sealer types, and between the root canal thirds. The effect sizes of the independent variables over the bond strength were calculated using the partial Eta squared (η^2 partial) test. The

program used for statistical analysis was SPSS 26.0 (IBM, Armonk, NY, USA) at $\alpha=5\%$.

Failure modes were descriptively reported in percentages regarding irrigation methods for each root canal sealer (AH-Plus and EndoSequence BC).

Results

Table 1 shows the bond strength results. There was a significant difference between the sealers ($P<0.001$), between final-step irrigation methods ($P<0.001$), and between the root canal thirds represented by the sliced specimens ($P<0.001$); however, the interaction between the variables presented no significance ($P>0.05$).

The AH-Plus sealer exhibited statistically higher bond strength than BC. Passive ultrasonic irrigation method for final-step irrigation exhibited higher bond strength values, and values were statistically significant when compared to the IC method. XP-endo Finisher had lower numerical bond strength values than PUI method and higher values than IC method. Bonding between the filling material and root canal dentinal walls statistically decreased in samples originating from the cervical third towards the apical third.

The calculation of effect sizes (confidence level of 95%, power of 80%, standard deviation, and a minimum difference to be detected between the groups in the mean-bond strength) of the independent variables over the bond strength showed that the types of sealers and the irrigation methods had similar effect sizes (0.124 vs. 0.147; $\alpha=5\%$). Root canal thirds had an effect size of 0.309 which means that the root third contributed to the variation of bond strength values in a range of 30.9%.

Most of the failure modes were mixed, regardless of the irrigation method or the sealer used (Table 2).

Table 1. Bond strength results (MPa) measured between root canal dentinal walls and root canal filling material, regarding the type of root canal sealer [AH-Plus (AH); EndoSequence BC Sealer (BC)], the final-step irrigation method [syringe-needle irrigation/conventional (IC); passive ultrasonic irrigation (PUI); XP-endo Finisher agitation (XP)], and the root canal thirds (cervical; middle; apical). *Root canal filling material was composed of root canal sealer and gutta-percha

Variable		Mean (SD)	P-value	η^2 partial
Root canal sealer	AH ^A	4.46 (2.24)	$P<0.001$	0.124
	BC ^B	3.47 (2.19)		
Final-step irrigation method	PUI ^A	4.52 (2.25)	$P<0.001$	0.147
	XP ^{AB}	3.93(3.93)		
	IC ^B	3.37(2.51)		
Root canal third	Cervical ^A	5.45 (2.39)	$p<0.001$	0.309
	Middle ^B	4.14 (1.99)		
	Apical ^C	2.30 (1.30)		

* ANOVA three-way and Tukey post-hoc tests $\alpha=5\%$; and Eta squared (η^2 partial) test.

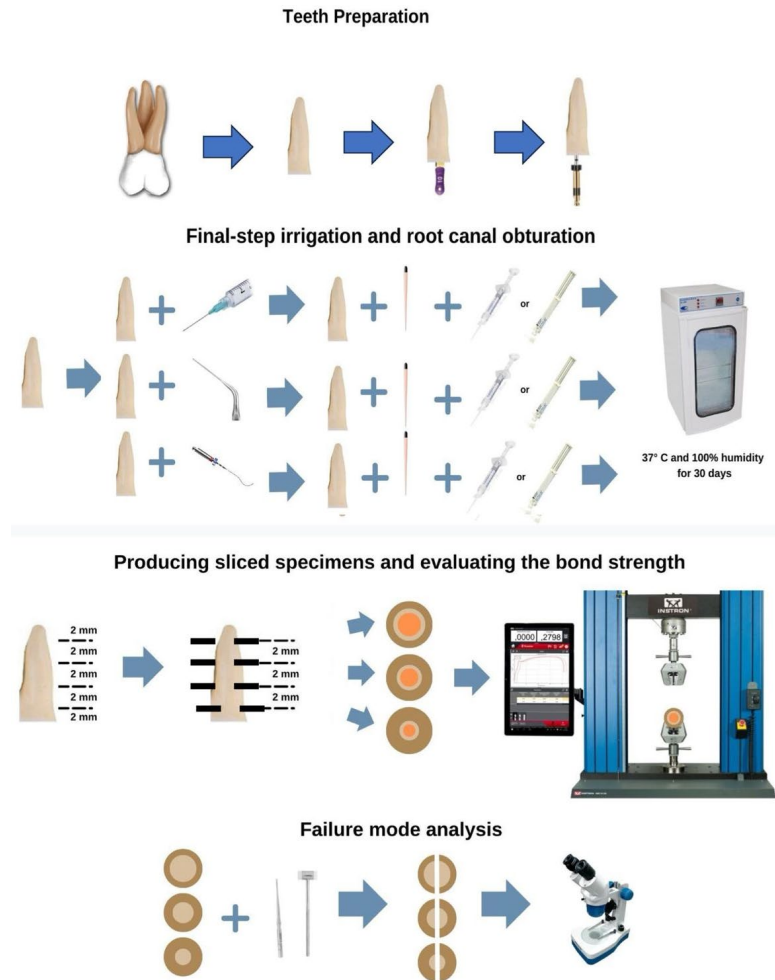


Figure 1. Graphical abstract showing the study protocol

Discussion

The results of this study indicate that the final-step irrigation method influence the bond strength of sealer to dentin. Both methods that used agitation of the irrigating solution (PUI and XP-endo Finisher agitation) exhibited better results than the IC method; however, contrary to our expectations, XP-endo Finisher was not the best agitation method. The type of sealer and the root thirds also influenced the bond strength, with AH-Plus being the best sealer, and the apical third exhibiting the least satisfactory results. Since no significance was found for the interaction between the variables, each variable (sealer, irrigation method, and root thirds) will be considered and discussed separately. The variable that contributed the most for variations in bonding strength was the root thirds, while the type of sealer and the method of irrigation contributed to a lesser extent.

Higher bond strength between filling material and dentin was observed from the agitation of 5 mL heated 17% EDTA and 5 mL

of 2.5% NaOCl. It is important to remember that the final-step irrigation without agitation (IC method) may lead to a vapor block caused by gases in the apical region; the space is constricted and unfavorable for the movement of the irrigating solution). This blocking effect hinders the penetration of the fluid to the apical region. When the solution was ultrasonically agitated, this undesirable effect was resolved, and the solution was pushed towards the apical third [39]. This improved cleaning may have increased the penetration of the sealers into the dentinal tubules and into other anatomical complexities. Although a correlation between bond strength and sealer penetration is still not proven [40], the literature has proven that

penetration properties, adaptation, and adherence pose a positive effect on the sealing, due to an increase in the area of contact between the sealer and the dentinal walls [41]. The justification for the XP group exhibiting lower sealer-dentin bond strength values, similar to the IC group is unavailable and needs further investigation. The XP-endo Finisher is a Nickel-

Titanium rotary instrument that expands up to 6 mm in diameter when rotating. The changes in instrument's cross-section improve the effectiveness by coming in contact with more areas of the root canal walls, improve irrigant distribution towards the apical third, and remove the remaining microbiota and smear layer after the biomechanical preparation [20, 21, 40, 41]. Despite of all these cited benefits, XP-endo Finisher agitation may lack the cavitation effect (bubble effect) produced by ultrasonic agitation.

Bond strength to dentin was higher for samples originating from the root canal obturated with AH-Plus sealer (4.46 MPa) in comparison to those obturated with EndoSequence BC sealer (3.47 MPa). Several previous studies have been investigating bond strength values in different dental materials and the values are fairly consistent. Some studies also reported higher bond strength values for AH [4, 8, 42-48].

It is common knowledge that the apical third is the most complex and critical area in the root canal system for instrumentation and obturation [35, 34]. In this study, the root third variable had the greatest effect on sealer bond strength, and the apical third presented the lowest values (mean/standard deviation of 2.30 ± 1.30 MPa), while the cervical and middle third presented 5.45 ± 2.39 MPa and 4.14 ± 1.99 MPa, respectively. Other studies also showed that sealer bond strength normally decreases in direction to the

apex [45, 49, 50]. The reduction in the density of the tubules from the cervical to the apical third may reduce the sealer penetration, due to the smaller diameter of the apical third tubules [33, 36].

A strong point of this study is that we had a single trained operator, experienced with the equipment and the experiment, which ensured standardization of the protocol. A limitation of this study is the possibility of not achieving the same results in clinical practice [51]. However, our results may guide dentists in selecting an irrigation method that uses agitation of the solution to improve bonding between canal walls and the filling material in the apical third, the most critical third of the root for cleaning.

Conclusion

This in vitro study found that using agitation on irrigating solution may improve the sealer bond strength to root canal walls. AH-Plus had the highest bond strength. The bond strength reduced significantly towards the apical third.

Declarations

Ethical approval and consent to participate: Study protocol was approved by CEUMA University Research Ethics Committee (protocol n 1.939.590).

Table 2. Failure modes (in percentages) regarding the final-step irrigation method [syringe-needle irrigation/conventional (IC); passive ultrasonic irrigation (PUI); XP-endo Finisher agitation (XP)] for each root canal sealer [AH-Plus (AH); EndoSequence BC Sealer (BC)]. *Root canal filling material was composed of root canal sealer and gutta-percha

Root canal sealer	Final-step irrigation method	Failure mode (%)
AH	PUI	Cohesive 19.35
		Mixed 51.61
		Adhesive 29.03
	XP	Cohesive 32.25
		Mixed 38.71
		Adhesive 29.03
	IC	Cohesive 31.03
		Mixed 48.27
		Adhesive 20.70
BC	PUI	Cohesive 13.80
		Mixed 75.86
		Adhesive 10.34
	XP	Cohesive 26.66
		Mixed 50.00
		Adhesive 23.33
	IC	Cohesive 17.40
		Mixed 60.87
		Adhesive 21.73

Availability of data and materials

All articles used to support the theory of this work are available on PubMed repository [<https://pubmed.ncbi.nlm.nih.gov/>]. The raw datasets generated and/or analyzed during this study are not publicly available, but are available from the corresponding author on reasonable request.

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Conflict of interest

None.

Author contributions:

Conceptualization: Trandafilov AAS, Carvalho CN. Data curation: Trandafilov AAS. Formal analysis: Trandafilov AAS, Carvalho CN, Filho EMM. Funding acquisition: Trandafilov AAS, Carvalho CN. Investigation: Trandafilov AAS. Methodology: Trandafilov AAS. Project administration: Carvalho CN, Bauer J, Filho EMM. Resources: Trandafilov AAS, Carvalho CN. Software: Filho EMM, Ferreira MC. Supervision: Carvalho CN. Validation: Carvalho CN, Filho EMM, Ferreira MC. Visualization: Carvalho CN, Filho EMM. Writing-original draft: Carvalho CN, Ferreira MC, Tavarez RRJ. Writing - review & editing: Carvalho CN, Ferreira MC, Tavarez RRJ, Filho EMM, Nogueira APA, Grazziotin-Soares R. All authors have read and agreed to the published version of the manuscript.

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