



Analysis of Bond Strength of Bioceramic Sealer Following Irrigation with Chlorhexidine

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Article Type: Original Article

Received: 11 Oct 2025

Accepted: 17 Dec 2025

Published: 28 Dec 2025

Doi: 10.22037/iej.v21i1.45971

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Abstract

Introduction: This study aimed to evaluate the bond strength of a bioceramic endodontic sealer and AH-Plus sealer irrigated with 2% chlorhexidine (CHX), compared to irrigation with 2.5% sodium hypochlorite (NaOCl) as a control group. **Materials and Methods:** Thirty freshly extracted single-rooted human teeth were prepared using reciprocating R25 and R40 files. Irrigation consisted of 2.5% NaOCl in the control group and 2% CHX gel in the experimental group, with additional irrigation using saline solution after each instrument change. Samples were divided into four groups: two with CHX, one with bioceramic sealer, and the other with AH-Plus sealer, and two with hypochlorite, one with bioceramic sealer and the other with AH-Plus sealer. The teeth were sectioned, and the resulting slices were subjected to the push-out test using a mechanical testing machine for measuring the microtensile force. After testing, samples were examined to determine the failure pattern. Data normality was assessed (Shapiro-Wilk, $P < 0.05$), and non-parametric analyses (Kruskal-Wallis with Dunn's post hoc, Wilcoxon rank-sum with Benjamini-Hochberg correction) were applied. Effect sizes (Cohen's d , Cliff's Delta), bootstrap 95% confidence intervals (CIs) (2000 replicates), and the post hoc power were calculated. Significance was set at $P < 0.05$. **Results:** AH-Plus exhibited higher bond strength than Sealer Plus BC across all root thirds, independent of irrigant. For Sealer Plus BC, bond strength was influenced by irrigant, with NaOCl producing higher values than CHX ($P < 0.05$). **Conclusion:** AH-Plus exhibited superior bond strength irrespective of the irrigant used. In contrast, the bond strength of the Sealer Plus bioceramic sealer was influenced by the irrigant, with NaOCl yielding higher values than CHX.

Keywords: Bioceramics; Chlorhexidine; Endodontics; Root Canal Sealer; Sodium Hypochlorite

Introduction

Endodontic treatment must be performed in accordance with technical, biological, and scientific principles that promote tooth preservation and create favorable conditions for periapical healing through immune system activity. Because endodontic sealers remain in close contact with apical periodontal tissues, the ideal material should be biocompatible and non-toxic [1].

An endodontic sealer is a plastic compound that complements root canal filling by occupying the space between gutta-percha cones and dentinal walls [2]. For clinical effectiveness, it should be easy to insert, provide an adequate working time, and exhibit

suitable physicochemical properties to ensure a proper seal. In addition, tolerance by periapical tissues is essential [2, 3].

Bioceramic sealers were introduced to improve the quality of root canal obturation. Composed of nanoscale particles, they can penetrate canal irregularities and dentinal tubules, promoting a chemical bond between dentin and the filling material [1]. These sealers are biocompatible, bioactive, and chemically stable, and they expand slightly during setting rather than shrink. Another notable property is the formation of hydroxyapatite during their hardening process, which enhances biological interaction [1, 3]. This feature distinguishes them from eugenol- and resin-based sealers, which lack bioactivity [4].



In parallel with sealers, irrigants are crucial for successful root canal therapy. Sodium hypochlorite (NaOCl) is the most widely used solution due to its ability to dissolve organic matter, remove the organic component of the smear layer, and act as an effective antimicrobial agent [5]. Chlorhexidine (CHX), in turn, is valued for its substantivity and long-lasting antimicrobial effect through binding to hydroxyapatite [6]. However, Zehnder [7] emphasized that CHX should not be considered a primary irrigant because it cannot dissolve necrotic tissue and shows lower efficacy against gram-negative bacteria compared to gram-positive ones.

When applied as a gel, CHX has demonstrated antimicrobial action against *Enterococcus faecalis*, and its viscous consistency may contribute to enhanced mechanical debridement of the canal system [8]. *In vitro* investigations, however, have reported conflicting outcomes regarding its effect on sealer adhesion: some found no influence [9], while others observed a reduction in bond strength [10].

Although CHX is frequently recommended as an irrigant, its interaction with radicular dentin and the adhesion of resin-based sealers is not fully understood [11]. Conversely, NaOCl, despite its effectiveness, may compromise adhesive procedures because its oxidizing effect leaves an oxygen-rich layer on dentin, potentially reducing bond strength and increasing microleakage [12].

To the best of our knowledge, neither the English nor Portuguese-language literature includes studies evaluating the use of CHX as an irrigant in combination with bioceramic sealers. This gap in knowledge motivated the present investigation. Therefore, this *in vitro* study aimed to evaluate bond strength using NaOCl (control), 2% CHX gel (Pharmacy Unifor, Fortaleza, Brazil), the bioceramic Sealer Plus BC (MK Life, Porto Alegre, Brazil), and AH-Plus (Dentsply DeTrey, Konstanz, Germany). The null hypothesis was that no significant difference in bond strength would be found between sealers, regardless of the irrigation protocol employed.

Materials and Methods

This study was approved by the Institutional Review Board of the University of Fortaleza under protocol number 5.991.483.

Sample calculation

A priori sample size was calculated using G*Power 3.1 (Heinrich-Heine-Universität, Düsseldorf, Germany) to detect differences in bond strength between two experimental groups: CHX+Bioceramic and CHX+AH-Plus. A two-tailed t-test ($\alpha=0.05$) with an effect size ($d=1.98$), derived from pilot data,

was used. The calculation indicated that 6 specimens per group (total $n=12$) would achieve 87% power. Although the a priori calculation suggested 6 specimens per group, additional samples were included to allow for control groups and potential specimen loss.

Sample delimitation

Thirty extracted human single-rooted teeth with single canals and fully formed apices were selected. Teeth presenting obliterated canals or pronounced curvatures were excluded. Specimens were stored at room temperature in distilled water to maintain hydration. The crowns were sectioned perpendicular to the long axis at the cemento-enamel junction using steel discs mounted on a straight handpiece, yielding root lengths between 14 and 16 mm.

Specimens were randomly allocated into four experimental groups. Groups 1 ($n=10$) and 2 ($n=10$) received CHX irrigation followed by obturation with gutta-percha and a bioceramic sealer or AH-Plus sealer, respectively. Groups 3 ($n=5$) and 4 ($n=5$) served as controls and received NaOCl irrigation followed by obturation with gutta-percha and either a bioceramic or AH-Plus sealer, respectively. The subdivision of the control specimens did not compromise statistical validity.

Root canal preparation

Canal exploration was performed using size 10, 21 mm K-files (Dentsply Maillefer, Ballaigues, Switzerland) to determine the working length, established 1 mm short of the apical foramen based on apical patency. Pre-enlargement was performed with size 15, 20, and 25 K-files at working length. Between each instrument, canals were irrigated with 2 mL of 2.5% NaOCl solution (Soda Clorada, Pharmacy Unifor, Fortaleza, Brazil).

In the 2% CHX gel group (Pharmacy Unifor, Fortaleza, Brazil), the canals were filled with the gel during instrumentation and flushed with 2 mL of saline solution at a constant rate for 1 min between each file change. After irrigation, the canals were refilled with fresh CHX gel. The saline rinse aimed to remove dentin debris suspended during root canal preparation.

Irrigation solutions were replenished with every instrument change, with total irrigation time standardized at 5 min. The gel was removed with 2 mL of sterile saline. Subsequently, canals were shaped with reciprocating R25 and R40 files (Reciproc, VDW, Munich, Germany) to establish the apical diameter. Irrigation with 17% EDTA (Iodontosul, Porto Alegre, Brazil) was performed, agitated manually with a needle and a gutta-percha cone, and maintained for 3 min. Canals were then rinsed with 1 mL of saline, dried with an endodontic aspirator, and completed with absorbent paper points.

The sealers used were an epoxy resin-based sealer (AH-Plus Jet, Dentsply DeTrey, Konstanz, Germany) prepared in a 1:1 ratio, and a Sealer Plus BC is a new calcium silicate-based sealer (bioceramic cement) (MK Life, Porto Alegre, Brazil).

The single-cone obturation technique was performed by fitting a gutta-percha cone with the same size and taper as the final shaping instrument to the full working length. The master cone was then coated with sealer and inserted to the working length, providing a snug fit at the apical constriction. Excess gutta-percha at the coronal portion was removed with a heated instrument, and vertical compaction was applied to adapt the material to the canal walls.

Access cavities were sealed with a temporary restorative material (Coltosol, Vigodent S/A Ind., Rio de Janeiro, Brazil). Specimens were stored for 30 days wrapped in sterile gauze moistened with saline to preserve hydration and sealer integrity. The gauze was replaced daily to ensure consistent humidity throughout the storage period.

Sample preparation

Roots were embedded in a low-melting-point material fixed onto an acrylic plate and mounted in a precision cutting machine (Isomet 1000). Serial sections were obtained using an extra-fine diamond blade at 200 rpm under continuous irrigation. After discarding the first millimeter of the cervical portion, three slices were obtained from each root: coronal, middle, and apical thirds, each approximately 1.5 mm thick. Slice thickness was confirmed with a digital caliper (Manual Steel Caliper 150 mm/6"; Mtx, China).

Before testing, calibration was conducted using five pilot specimens to standardize plunger positioning and force application. Intra-examiner reliability was assessed by repeating the push-out test in 20% of specimens after two weeks. The intraclass correlation coefficient (ICC) was calculated, with values ≥ 0.80 considered indicative of excellent reproducibility.

The specimens were positioned with the cervical portion facing downward on a support matrix containing a 3.5 mm aperture for stabilization. A compressive force of 0.5 mm/min was applied using a rod attached to the testing machine (Microtensile OM100, Odeme Dental Research, Luzerna, SC, Brazil) fitted with an extra-fine 0.8 mm tip. The tip was positioned at the center of the specimen, corresponding to the filling material. Push-out testing provided bond strength values initially recorded in grams (g), which were converted to Newtons (N) using the factor $1\text{ g}=0.009807\text{ N}$ and subsequently expressed in megapascals (MPa). The formulas used for bond strength and area calculations are presented in Table 1, following Marcos *et al.* [13] and Campelo *et al.* [14]. This

method has been shown to yield results equivalent to the standard truncated cone surface area calculation, ensuring accurate bond strength measurements in MPa.

After push-out testing, specimens were examined under a dental operating microscope (Alliance, São Carlos, Brazil) at 25 \times magnification to classify failure patterns as: adhesive (separation at the dentin/sealer interface), cohesive (fracture within the sealer), or mixed (combination of adhesive and cohesive failure).

Statistical analysis

Data were tabulated in Microsoft Excel (Microsoft, Redmond, WA, USA) and analyzed using IBM SPSS Statistics version 22 (IBM, Armonk, NY, USA). Normality of the data was assessed using the Shapiro–Wilk test ($P<0.05$), which indicated non-normal distribution; therefore, non-parametric tests were applied. Group comparisons were performed using the Kruskal–Wallis test, followed by Dunn’s post hoc test for multiple pairwise comparisons and Wilcoxon rank-sum tests with Benjamini–Hochberg correction for exact pairwise comparisons. These non-parametric tests are appropriate for small sample sizes, unequal group distributions, and do not assume homogeneity of variances. The significance level was set at 5%. Effect sizes were calculated using Cohen’s *d* and Cliff’s *Delta* to quantify the magnitude of differences between groups and root thirds. Bootstrap resampling (2000 replicates) was applied to estimate robust 95% confidence intervals (CIs) for mean bond strength values. Post hoc statistical power was computed to evaluate the probability of detecting true effects given the small sample size.

Results

To evaluate bond strength in gutta-percha obturations and compare mean values across root thirds, two sealers (AH-Plus and Sealer Plus BC) were tested with two irrigants (2% CHX gel and 2.5% NaOCl). Data were non-normal (Shapiro–Wilk, $P<0.05$), so non-parametric tests were applied. In Tables 2-5, different letters indicate statistically significant differences, and Appendix 1 provides quartile-based analysis.

The Kruskal–Wallis test revealed significant differences among the four groups ($\chi^2=45.82$, $df=3$, $P<0.001$), showing that at least one group differed. Pairwise Wilcoxon tests adjusted by Benjamini–Hochberg confirmed very significant differences for Groups 1 \times 2 and 1 \times 4 ($P<0.001$), significant differences for Groups 2 \times 3 ($P=0.001$) and 3 \times 4 ($P=0.0004$), and no significant differences for Groups 3 \times 1 ($P=0.07$) and 2 \times 4 ($P=0.09$). Overall, Groups 1, 2, and 4 were significantly different, while Group 3 had intermediate values (Table 6).

Table 1. Parameters and formulas used to convert force (N) into mean bond strength (MPa)

Parameter	Formula
Mean Bond Strength	Strength (N)/Area (mm ²)
Area	$A=\pi(R+r)\sqrt{(h^2+(R-r)^2)}$

π : 3.14, r_1 : larger radius, r_2 : smaller radius, h : thickness

Table 2. Comparison of mean bond strength (MPa) and standard deviation among groups in the cervical third group

Group	Root cervical third-pressure values in Mpa			
	Mean (SD)	Minimum	Maximum	n
CLX-AH-Plus	5.27 (1.9) ^a	0.51	7.63	10
Hypo-AH-Plus (control)	4.80 (2.09) ^a	2.34	6.98	5
CLX-Sealer Plus BC	2.03 (0.84) ^b	0.19	3.97	10
Hypo-Sealer Plus BC (control)	3.28 (2.15) ^{ab}	1.77	7.51	5

CLX: 2% chlorhexidine gel, Hypo: 2.5% sodium hypochlorite, BioC: bioceramic Sealer Plus BC. Different letters indicate significant differences ($P<0.05$) by Dunn's test in each group

Table 3. Comparison of mean bond strength (MPa) and standard deviation among groups in the middle third

Group	Root middle third-values in Mpa			
	Median	Minimum	Maximum	n
CLX-AH-Plus	4.97 (1.77) ^a	0.46	6.41	10
Hypo-AH-Plus (control)	6.45 (1.83) ^a	5.62	10.08	5
CLX-Sealer Plus BC	0.62 (0.35) ^b	0.06	2.70	10
Hypo-Sealer Plus BC (control)	1.41 (1.2) ^{ab}	0.16	3.41	5

CLX: 2% chlorhexidine gel, Hypo: 2.5% sodium hypochlorite, BioC: bioceramic Sealer Plus BC. Different letters indicate significant differences ($P<0.05$) by Dunn's test in each group

Table 4. Comparison of mean bond strength (MPa) and standard deviation among groups in the apical third

Group	Root apical third-values in Mpa			
	Median	Minimum	Maximum	n
CLX-AH-Plus	5.68 2.52) ^a	2.52	10.13	10
Hypo-AH-Plus (control)	6.70 (4.4) ^a	3.73	14.87	5
CLX-Sealer Plus BC	0.49 (0.80) ^b	0.19	5.97	10
Hypo-Sealer Plus BC (control)	1.38 (0.87) ^{ab}	0.38	4.64	5

CLX: 2% chlorhexidine gel, Hypo: 2.5% sodium hypochlorite, BioC: bioceramic Sealer Plus BC. Different letters indicate significant differences ($P<0.05$) by Dunn's test in each group

Table 5. Distribution of failure modes in the study group

Group	Fracture pattern		
	Adhesive	Cohesive	Mixed
CLX-Sealer Plus BC	20%	70%	10%
CLX-AH-Plus	30%	44%	26%
Hypo-Sealer Plus BC	–	100%	–
Hypo-AH-Plus	33%	67%	–

CLX: 2% chlorhexidine gel, HYPO: 2.5% sodium hypochlorite, BioC: bioceramic Sealer Plus BC

Table 6. Pairwise comparisons of bond strength among experimental groups

Comparison	P-value	Significance
Comparison	0.000	***
Groups 1 × 2	0.000	***
Groups 1 × 4	0.001	**
Groups 2 × 3	0.0004	**
Groups 3 × 4	0.07	Not significant
Groups 3 × 1	0.09	Not significant

Significance thresholds: ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; Not significant: $P\geq 0.05$

AH-Plus consistently showed the highest bond strength, especially when combined with NaOCl, which outperformed CHX. Sealer Plus BC showed lower bond strength regardless of irrigant. NaOCl slightly improved Sealer Plus BC compared to CHX, but values remained below those of AH-Plus. In the apical third, Sealer Plus BC was weaker than AH-Plus for both irrigants, while AH-Plus was not significantly affected by irrigant type ($P>0.05$). When considering median values across all thirds, AH-Plus was significantly stronger than Sealer Plus BC ($P<0.05$).

No significant differences were observed among cervical, middle, and apical thirds (Kruskal–Wallis $\chi^2=0.614$, $df=2$, $P=0.736$; pairwise $P=0.77$). The effect size between Groups 1 and 2 was large (Cohen's $d=-1.98$; 95% CI [-2.61, -1.35]), while differences between cervical and apical thirds were negligible (Cliff's $\delta=0.044$). Bootstrap analysis with 2000 replicates estimated the 95% bias-corrected and accelerated confidence interval for mean bond strength as 3.23–4.32, giving a reliable estimate that does not rely on normality assumptions. Post hoc power of 39.5% suggests the study was sufficient to detect these large observed effects, but would have limited sensitivity for smaller differences.

Fracture patterns reflected the material and irrigant combinations. In the CHX group with Sealer Plus BC, 20% of failures were adhesive, 70% cohesive, and 10% mixed, whereas with AH-Plus, 30% were adhesive, 44% cohesive, and 26% mixed. In the NaOCl group with Sealer Plus BC, all failures were cohesive. with AH-Plus and NaOCl, 33% were adhesive and 67% cohesive (Table 5).

In summary, despite the limited sample size, the combined use of non-parametric statistics, effect size measures, and bootstrap confidence intervals allowed for reliable inference. The large observed effects (Cohen's $d>1.5$) indicate that the significant differences are unlikely due to sampling variability. Smaller effects would require larger sample sizes for reliable detection.

Discussion

Adhesion is the force that holds two substances together through molecular attraction when placed in close contact [15]. In this study, push-out tests were employed to evaluate the adhesion of different endodontic sealers to root dentin, as this method allows assessment at a site of direct clinical relevance. Unlike tests involving coronal dentin proposed in previous studies [16, 17], this approach reflects more realistic clinical conditions.

Previous research has assessed adhesion across different root thirds [18, 19], yet consensus is lacking regarding which third exhibits superior adhesion, with some studies favoring the

coronal third [20] and others, the apical third [21]. Given the clinical importance of effective adhesion throughout the canal, each third was analyzed independently in the present study.

The results indicated that the resin-based sealer AH-Plus exhibited significantly higher bond strength in the apical third compared to the bioceramic sealer Sealer Plus Bioceramic, leading to rejection of the null hypothesis. Overall differences between the main experimental groups were statistically significant ($P<0.001$). Pairwise analyses showed that this effect was driven by significant differences involving specific group comparisons, whereas other pairwise contrasts did not reach statistical significance, as detailed in Table 6. No significant differences were observed among cervical, middle, and apical root thirds ($P=0.736$). These findings are consistent with Shieh *et al.* [22], who also reported greater bond strength of AH-Plus in the apical third, while contrasting reports in the literature describe either higher bond strength for bioceramic sealers or comparable performance between resin-based and bioceramic sealers, particularly in the middle and cervical thirds [23–25].

The superior performance of AH-Plus is likely attributable to its epoxy resin composition, which allows chemical interaction with amino groups in dentinal collagen [23]. This mechanism is particularly relevant in the apical third, where sealing is critical due to the presence of periapical fluids. As the cervical and middle thirds are often involved in post placement, a reliable apical seal becomes even more essential.

Bonding to dentin remains challenging due to its high-water content, density, and variation in tubule structure along the root canal. The apical third is the most challenging region owing to reduced tubule diameter, lower density, and increased sclerotic dentin, emphasizing the need for an effective apical seal [21, 24]. Effect size calculations confirmed the magnitude of these differences (Cohen's $d=-1.98$), while bootstrap confidence intervals supported robust mean bond strength estimates (95% CI 3.23–4.32).

The use of 0.2% CHX solution for 1 min has been shown to produce the highest bond-strength values when compared with 5% sodium hypochlorite before the application of a self-cured resin cement [25]. Moreover, residual NaOCl or CHX has not been reported to adversely affect the bond strength of resin-based sealers [26]. However, this was not confirmed in the present study, in which the findings indicate that AH-Plus promotes superior bond strength irrespective of the irrigant employed. In contrast, for the Bioceramic sealer, the type of irrigant exerted a significant influence, with NaOCl proving more favourable than CHX.

The use of 2.5% NaOCl was justified by its antimicrobial activity [27] and the capacity to dissolve organic tissue.

Although widely adopted, NaOCl contributes to smear layer formation, necessitating final irrigation with EDTA, which was applied in this study [28]. CHX gel at 2% concentration was employed due to its strong antibacterial effect, diffusion capacity, and substantivity, providing sustained antimicrobial action [26]. Our results showed that the use of AH-Plus resulted in higher bond strength values, particularly in the NaOCl group, which outperformed the CHX group. In contrast, the groups filled with the bioceramic sealer Sealer Plus BC showed substantially lower values, regardless of the irrigant used.

Razmi *et al.* [29] reported that AH-Plus bond strength was unaffected by the type of irrigant, in agreement with our findings. However, they also noted reduced bond strength when CHX was used with a bioceramic sealer (EndoSequence BC), which was not replicated here. These authors further observed that AH-Plus performed better in dry canals, whereas moisture did not significantly influence bioceramic sealers, supporting the use of paper points for drying in the current protocol. Regarding fracture patterns, mixed and cohesive failures predominated for AH-Plus, while Sealer Plus BC mainly exhibited cohesive failures, indicating satisfactory bonding of both sealers to dentin [29].

Limitations

This study has some limitations. A fundamental limitation of this study's design is the comparison of CHX in a gel formulation to NaOCl as a liquid. The pronounced difference in their rheological properties, such as flow, penetration into dentinal tubules, and interaction with the smear layer, represents a significant confounding variable. Consequently, the observed effects on sealer bond strength cannot be attributed solely to the chemical composition of the irrigants (CHX vs. NaOCl) and are inherently linked to their physical state. Our conclusions regarding irrigant influence should therefore be interpreted with this key constraint in mind.

Furthermore, while an a priori power calculation was performed, the post-hoc power remained low (39.5%). This indicates that the study was adequately powered to detect the large effect sizes we observed for the primary comparisons, such as between sealers, but was underpowered to detect more subtle effects or to fully explore all interactions within our complex multi-group, multi-level (root third) design. The small sample size in the control groups ($n=5$) further limits the robustness of inferences for those specific comparisons. Thus, while our main findings are compelling, future studies with larger sample sizes are warranted to confirm these results and investigate potential smaller, yet clinically relevant, differences.

Overall, the findings should be validated by randomized clinical trials. The type of endodontic sealer plays a key role in

the long-term success of root canal treatment, with bioceramic sealers being particularly indicated in specific clinical situations such as open apices.

Conclusion

AH-Plus exhibited superior bond strength irrespective of the irrigant used. In contrast, the bond strength of the Sealer Plus bioceramic sealer was influenced by the irrigant, with NaOCl yielding higher values than CHX.

Acknowledgments

The authors gratefully acknowledge the support of colleagues from the Professional Master's Program in Dentistry and the undergraduate Dentistry course at the University of Fortaleza (UNIFOR). The authors further express sincere gratitude to UNIFOR for funding this publication.

Conflict of interest

None.

Funding support

This publication was funded by the University of Fortaleza, Edson Queiroz Foundation.

Authors' contributions

Conceptualization: JESF/BAA/EDGF; Methodology: JESF/BAA/FAG/EDGF; Formal Analysis and Investigation: LGC/JMS/JESF/EDGF; Writing-Original Draft Preparation: LGC; Writing-Review and Editing: JMS/ JESF/BAA/FAG/EDGF; Supervision: EDGF; All authors read and approved the final manuscript.

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Please cite this paper as: Costa LG, Saldanha JM, da Silva-Filho JE, Aguiar BA, de Almeida Gomes F, Gurgel-Filho ED. Analysis of Bond Strength of Bioceramic Sealer Following Irrigation with Chlorhexidine. *Iran Endod J.* 2026;21(1): e4. Doi: 10.22037/iej.v21i1.45971.

Appendix 1. Quartile-based statistical analysis

Group	ID	Force	Q1	Q3	IQR	Lower Bound	Upper Bound	Outlier
1	C1	0.192	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M1	0.581	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A1	1.302	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C2	1.828	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M2	0.056	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A2	0.02	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C3	1.678	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M3	0.111	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A3	5.971	0.3595	2.335	1.9755	-2.60375	5.29825	Yes
1	C4	3.911	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M4	2.546	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A4	2.342	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C5	3.965	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M5	0.532	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A5	0.548	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C6	2.224	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M6	1.275	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A6	2.694	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C7	3.718	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M7	1.755	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A7	0.316	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C8	0.436	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M8	2.696	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A8	0.193	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C9	1.277	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M9	0.225	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A9	0.433	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	C10	2.314	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	M10	0.661	0.3595	2.335	1.9755	-2.60375	5.29825	No
1	A10	0.335	0.3595	2.335	1.9755	-2.60375	5.29825	No
2	C1	4.955	3.957	6.257	2.3	0.507	9.707	No
2	M1	2.985	3.957	6.257	2.3	0.507	9.707	No
2	A1	2.518	3.957	6.257	2.3	0.507	9.707	No
2	C2	3.657	3.957	6.257	2.3	0.507	9.707	No
2	M2	3.932	3.957	6.257	2.3	0.507	9.707	No
2	A2	7.818	3.957	6.257	2.3	0.507	9.707	No
2	C3	3.452	3.957	6.257	2.3	0.507	9.707	No
2	M3	5.377	3.957	6.257	2.3	0.507	9.707	No
2	A3	4.032	3.957	6.257	2.3	0.507	9.707	No
2	C4	0.506	3.957	6.257	2.3	0.507	9.707	Yes
2	M4	0.463	3.957	6.257	2.3	0.507	9.707	Yes
2	A4	4.49	3.957	6.257	2.3	0.507	9.707	No
2	C5	5.498	3.957	6.257	2.3	0.507	9.707	No
2	M5	5.746	3.957	6.257	2.3	0.507	9.707	No
2	A5	10.131	3.957	6.257	2.3	0.507	9.707	Yes
2	C6	6.384	3.957	6.257	2.3	0.507	9.707	No
2	M6	4.349	3.957	6.257	2.3	0.507	9.707	No

2	A6	6.75	3.957	6.257	2.3	0.507	9.707	No
2	C7	6.203	3.957	6.257	2.3	0.507	9.707	No
2	M7	6.412	3.957	6.257	2.3	0.507	9.707	No
2	A7	2.872	3.957	6.257	2.3	0.507	9.707	No
2	C8	5.037	3.957	6.257	2.3	0.507	9.707	No
2	M8	4.563	3.957	6.257	2.3	0.507	9.707	No
2	A8	6.303	3.957	6.257	2.3	0.507	9.707	No
2	C9	7.628	3.957	6.257	2.3	0.507	9.707	No
2	M9	6.114	3.957	6.257	2.3	0.507	9.707	No
2	A9	5.093	3.957	6.257	2.3	0.507	9.707	No
2	C10	5.702	3.957	6.257	2.3	0.507	9.707	No
2	M10	5.681	3.957	6.257	2.3	0.507	9.707	No
2	A10	6.275	3.957	6.257	2.3	0.507	9.707	No
3	C1	3.278	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	M1	2.05	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	A1	2.91	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	C2	7.505	1.195	3.3185	2.1235	-1.99025	6.50375	Yes
3	M2	5.417	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	A2	4.642	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	C3	3.359	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	M3	0.828	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	A3	1.012	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	C4	3.277	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	M4	0.158	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	A4	0.38	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	C5	1.766	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	M5	1.409	1.195	3.3185	2.1235	-1.99025	6.50375	No
3	A5	1.378	1.195	3.3185	2.1235	-1.99025	6.50375	No
4	C1	4.805	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	M1	10.078	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	A1	14.871	4.5415	7.621	3.0795	-0.07775	12.24025	Yes
4	C2	6.7	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	M2	6.238	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	A2	6.697	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	C3	2.996	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	M3	5.62	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	A3	8.264	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	C4	2.343	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	M4	6.451	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	A4	4.278	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	C5	6.978	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	M5	8.284	4.5415	7.621	3.0795	-0.07775	12.24025	No
4	A5	3.729	4.5415	7.621	3.0795	-0.07775	12.24025	No

Abbreviations: ID, Sample Identification; Q1, First quartile; Q3, Third quartile; IQR, Interquartile range. **Legends:** 1 – Group 1, Chlorhexidine and Bioceramic sealer; 2 – Group 2, Chlorhexidine and AH Plus; 3 – Group 3, Sodium hypochlorite and Bioceramic sealer; 4 – Group 4, Sodium hypochlorite and AH Plus.

Thirids: C, Cervical third; M, Middle third; A, Apical third.