

Microhardness of Root Canal Sealers at Different Canal Thirds: a Pilot *In Vitro* Study

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ABSTRACT

Objectives: The aim of this *in vitro* study was to evaluate the microhardness of various root canal sealers in the coronal, middle and apical thirds of the simulated root canals.

Material and Methods: The study analysed the bioceramic root canal sealers (RCS) TotalFill® BC Sealer™, AH Plus® Bioceramic Sealer and BioRoot™ RCS, the resin-based RCS AH Plus® Jet™ and the bioceramic cement ProRoot® MTA (positive control). Gypsum samples simulating root canals were prepared and divided into five experimental groups. The canals were filled with the respective RCS and incubated for 24 hours at 37 °C (95% humidity). The microhardness of the RCS in different thirds of the canal was evaluated using the Vickers microhardness (HV) test.

Results: The microhardness ranged from 6.8 HV (AH Plus® Bioceramic Sealer) to 70.3 HV (ProRoot® MTA). TotalFill® BC Sealer™ remained stable across all thirds ($P > 0.05$), AH Plus® Bioceramic Sealer was significantly harder in the coronal third ($P = 0.008$), BioRoot™ RCS and ProRoot® MTA were significantly harder in the apical third ($P < 0.05$), while AH Plus® Jet™ showed significantly higher hardness in the middle and apical thirds ($P = 0.008$). BioRoot™ RCS showed no significant difference in microhardness compared to ProRoot® MTA ($P = 0.146$). Other RCS were significantly less hard than the positive control ($P < 0.05$).

Conclusions: Sealer microhardness varied across canal thirds, with TotalFill® BC Sealer™ showing stability, BioRoot™ RCS resembling the positive control and the others displaying regional variation.

Keywords: endodontics; hardness tests; *in vitro*; root canal filling materials.

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INTRODUCTION

Successful root canal treatment aims to eradicate or substantially diminish the microbial load within the root canal system and to prevent reinfection by ensuring proper chemo-mechanical preparation and hermetic sealing of the canal with a final filling material [1]. Achieving high-quality canal obturation depends on the selection of an endodontic sealer that ensures a tight seal, demonstrates biocompatibility and offers resistance to mechanical stress.

Modern endodontics offers a wide variety of root canal sealers (RCS) which vary in chemical composition and physical properties. In recent years, there has been an increasing focus on bioceramic-based RCS due to their advanced biological compatibility, bioactivity and favourable physical characteristics. These materials have evolved through several technological generations - from the initial mineral trioxide aggregate (MTA) based cements to the latest pre-mixed, thermally stable and bioactive RCS [2].

Bioceramic RCS are recognised for their excellent biological compatibility, antibacterial properties, mechanical strength, strong bond to the tooth's hard tissues and ability to initiate cementogenesis in the apical region of the root [3,4]. Meanwhile, the resin-based RCS AH Plus® Jet™ (Dentsply DeTrey GmbH; Konstanz, Germany) also exhibits excellent physical properties and is still considered the «gold standard» in endodontics [5]. The setting process of bioceramic RCS necessitates moisture [6], yet literature suggests that moisture levels within the thirds of the root canal may vary. The apical region is usually characterised by elevated levels of humidity, in contrast to the drier conditions that prevail in the coronal portion [7]. Furthermore, recent evidence suggests growing clinical use of single-cone obturation with bioceramic sealers, supported by laboratory studies showing favourable physicochemical and biological properties, and by clinical systematic reviews reporting outcomes comparable to traditional techniques [8,9]. Therefore, the setting and stability of these materials have been the focus of extensive research [10] due to their potential influence on the long-term effectiveness and prognosis of the treatment.

Microhardness testing is a method of assessing the sealer's setting process and how it changes under different conditions [11]. Recent studies have examined the alterations in the microhardness of RCS under diverse environmental conditions [12,13]. The physical properties of bioceramic RCS are susceptible to variation in moisture conditions [7]. Different levels of the intraradicular moisture had

a significant effect on the filling quality of bioceramic RCS with the single-cone technique [14]. These variations may lead to differences in material setting and adaptation in different regions of the root canal, potentially affecting the long-term sealing ability and durability of the obturation. However, there is still a lack of studies examining microhardness variations of RCS in different regions of the root canal. Therefore, the aim of this pilot *in vitro* study is to evaluate the microhardness of different root canal sealers in the coronal, middle and apical thirds of the simulated root canal.

Study hypothesis - the microhardness of root canal sealers varies between different thirds of the root canal.

MATERIAL AND METHODS

The present study was conducted at the Department of Dental and Oral Pathology, Lithuanian University of Health Sciences, Lithuania, and the Faculty of Mechanical Engineering and Design, Kaunas University of Technology, Lithuania, from February 1, 2024 to February 1, 2025, in accordance with the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) guidelines [15] (Figure 1).

Sample preparation

The characteristics of the tested materials are presented in Table 1. Five different materials were tested in this study:

- TotalFill® BC Sealer™ bioceramic canal filling paste (FKG Dentaire SA; La Chaux-de-Fonds, Switzerland);
- AH Plus® Bioceramic Sealer (Dentsply Sirona; Ballaigues, Switzerland);
- BioRoot™ RCS bioactive mineral root canal sealer (Septodont; Saint-Maur-des-Fossé, France);
- AH Plus® Jet™ root canal sealer (Dentsply DeTrey GmbH; Konstanz, Germany);
- ProRoot® MTA root repair material (Dentsply Tulsa Dental Specialties; Johnson City, Tennessee, USA), as a positive control.

For each RCS cylindrical moulds measuring 15 mm in length, with an access diameter of 2 mm and an apical foramen diameter of 0.2 mm were prepared using a tapered drill and type IV gypsum, Japan-Stone (Siladent Dr. Böhme & Schöps GmbH; Goslar, Germany), following the recommendations of ISO 6876/2012, to ensure proper hydration of the materials during setting [6]. Prior to canal filling, the moulds were immersed in room temperature water for 24 hours. This preliminary hydration step was imperative

because bioceramic RCS necessitate moisture for optimal setting. It is noteworthy that the specimens designated for AH Plus® Jet™ did not undergo pre-hydration, as this step is not necessary for the setting reaction of this material. The random allocation of

samples into groups was not applied, as all models were identical and featured standardized root canal morphology. The samples were divided into five experimental groups. All tests were conducted in triplicate.

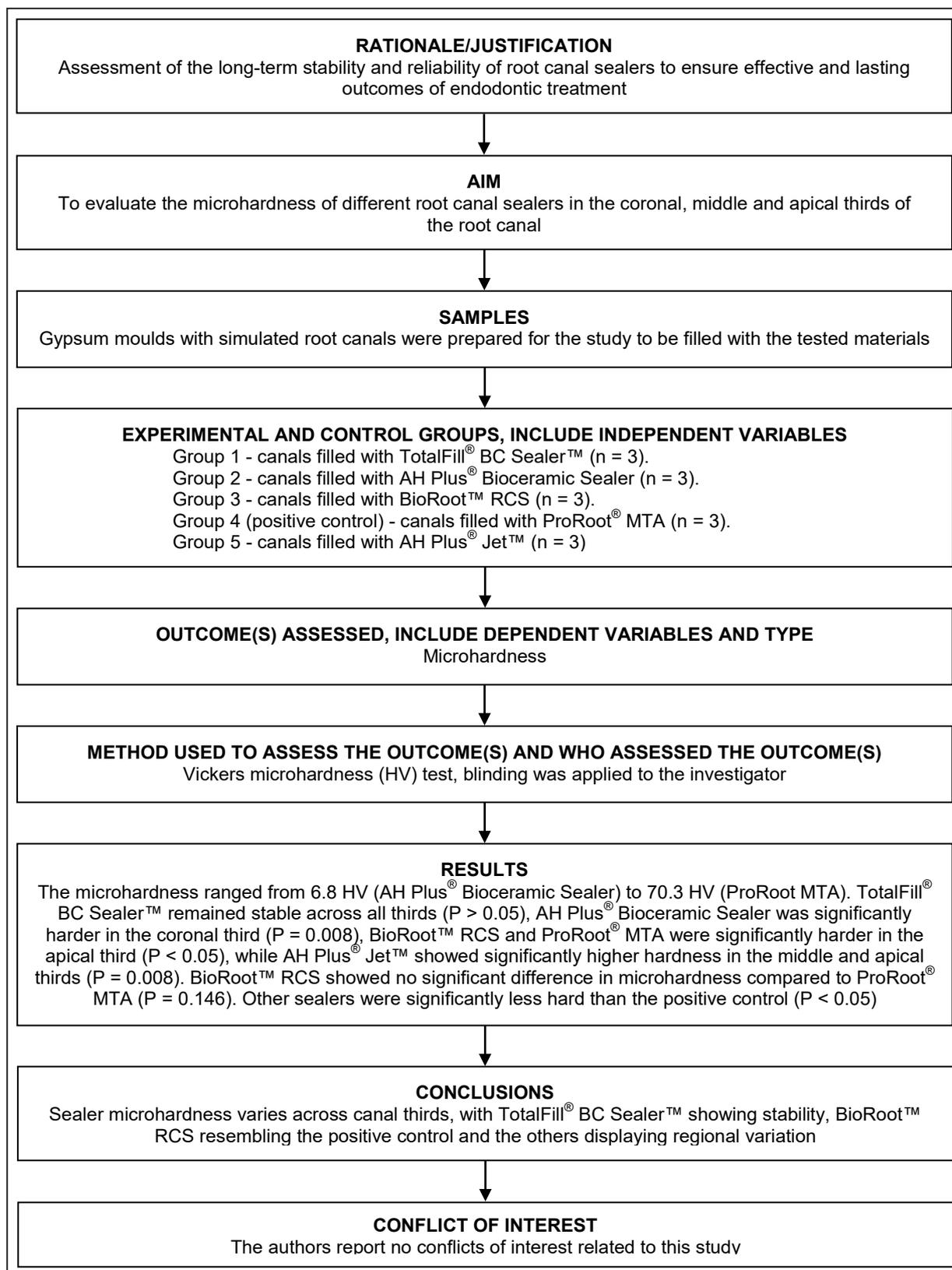


Figure 1. Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) flowchart.

Table 1. Materials used in the study

Material	Chemical composition	Manufacturer
TotalFill® BC Sealer™	Zirconium oxide, calcium silicate, calcium phosphate, calcium hydroxide, thickening agent	FKG Dentaire SA; La Chaux-de-Fonds, Switzerland
AH Plus® Bioceramic Sealer	Zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, thickening agent	Dentsply Sirona; Ballaigues, Switzerland
BioRoot™ RCS	Powder: tricalcium silicate, zirconium oxide, povidone. Liquid: calcium chloride, polycarboxylate	Septodont; Saint-Maur-des-Fossé, France
AH Plus® Jet™	Paste A: bisphenol-A epoxy resin, Bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: dibenzyl diamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil	Dentsply DeTrey GmbH; Konstanz, Germany
ProRoot® MTA (positive control)	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum, calcium oxide, bismuth oxide	Dentsply Tulsa Dental Specialties; Johnson City, Tennessee, USA

The root canal obturation procedure was performed under the operating microscope (Carl Zeiss Meditec Inc., Switzerland) at x12.5 magnification to ensure optimal quality control. TotalFill® BC Sealer™ and AH Plus® Bioceramic Sealer were injected directly into the moulds. BioRoot™ RCS, AH Plus® Jet™ and ProRoot® MTA were mixed according to manufacturers' instructions and inserted into the moulds.

The samples of bioceramic materials were covered with moist gauze and placed in polyethylene bags to maintain moisture levels. All samples incubated in a humidity chamber at 37 °C (95% humidity) for 24 hours to allow setting.

Vickers microhardness (HV) test

After setting, the samples were horizontally sectioned at 5 mm intervals to represent the coronal, middle and apical thirds of the canal. The microhardness of the RCS in different thirds of the canal was measured using the Vickers hardness testing machine - Mitutoyo HM-220® B-type (Mitutoyo Europe GmbH; Neuss, Germany). The study applied the blinding principle - the researcher performing the measurements was unaware of the material used in each specific sample. The surface of each sample was polished using abrasive papers of varying grit sizes from P800 to P3000 (Riken Corundum Co., Ltd.; Konosu, Japan) and final polishing was performed using diamond suspension AquaPol-M (Kemet International Ltd.; Maidstone, United Kingdom). A load of 0.05 N was applied for 10 seconds for microhardness assessment. Measurements were taken on the Vickers scale using a pyramid-shaped indenter. For each canal surface, three indentations were performed and arranged as an equilateral triangle within the 2 mm diameter canal

cross-section. The sides of the triangle measured approximately 1.5 mm, ensuring that the indentations were evenly spaced from each other and from the canal edges. All indentations were visually checked and any measurements showing irregularities (e.g., cracks or incomplete impressions) were excluded. The horizontal and vertical dimensions of the indentations were measured using the software AVPAK-20 V3.0 (Mitutoyo Europe GmbH), from which the hardness values were calculated according to the Vickers methodology.

Statistical analysis

The collected study data were analysed using the statistical software IBM SPSS® Statistics software version 30.0 (IBM Corp.; Armonk, New York, USA). As the data did not meet the normality criteria, they were presented as median and interquartile range (IQR). The Kruskal-Wallis test followed by Dunn's post-hoc test was used to assess differences between independent samples and the Wilcoxon signed-rank test was applied for dependent samples analysis. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The microhardness of the RCS in the coronal, middle and apical thirds of the canal, 24 hours after material mixing, is shown in Figure 2.

Differences in the microhardness of the tested sealers in different thirds of the canal

TotalFill® BC Sealer™ showed median microhardness

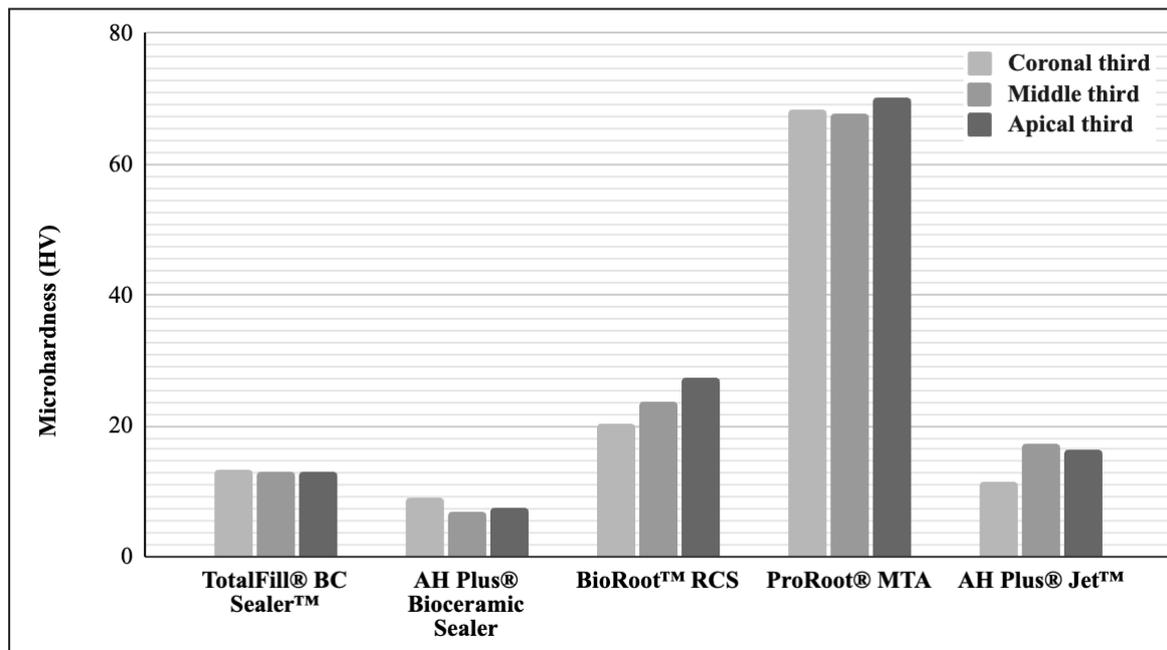


Figure 2. Microhardness of the materials in different thirds of the canal.

values from 13.1 to 13.2 HV with no significant differences between thirds ($P = 0.621$, $P = 0.866$, $P = 0.944$, respectively). The microhardness of AH Plus® Bioceramic Sealer ranged from 6.8 to 8.9 HV with the coronal third significantly harder than the middle and apical thirds ($P = 0.008$). BioRoot™ RCS displayed median microhardness from 20.4 to 27.3 HV, with a significant increase from coronal to middle ($P = 0.008$) and middle to apical ($P = 0.008$). The microhardness of ProRoot® MTA ranged from 67.8 to 70.3 HV, with the apical third significantly harder than coronal and middle thirds ($P < 0.05$). However, no statistically significant difference was observed between the coronal and middle thirds ($P = 0.889$). AH Plus® Jet™ microhardness varied from 11.6 to 17.2 HV, with the coronal third significantly less hard than middle and apical ($P = 0.008$), while there was no significant difference in microhardness between the middle and apical thirds ($P = 0.722$) (Figure 3).

Comparison of the microhardness of different root canal sealers

The microhardness of the tested sealers ranged from 6.8 HV (AH Plus® Bioceramic Sealer in the middle third) to 70.3 HV (ProRoot® MTA in the apical third) (Table 2).

In all thirds, the microhardness of BioRoot™ RCS did not differ significantly from the positive control ($P = 0.146$), while TotalFill® BC Sealer™, AH Plus® Bioceramic Sealer and AH Plus® Jet™ sealers were significantly less hard ($P < 0.05$).

DISCUSSION

The selection of an appropriate RCS is one of the essential components of the endodontic treatment process. The physical and chemical properties of the sealer determine the sealing of the canal, the material's biological compatibility and resistance to mechanical stress [16].

A number of studies have previously been conducted on the microhardness of RCS, employing shallow plastic or metal cylindrical samples [12,17]. The utilisation of such samples facilitates the standardization of results across different studies. However, it does not reflect clinical situations where sealers are placed deep within the canal. It is hypothesised that the microhardness values of bioceramic RCS, the setting process of which is moisture-dependent, may vary at different canal depths due to uneven moisture distribution in different areas of the root canal. This study aimed to investigate the microhardness of RCS at different canal depths, closely mimicking the clinical situation. In previous research, cylindrical moulds have been used to study the properties of RCS at different depths [18,19]. In accordance with ISO 6876/2012 standards, materials necessitating the presence of moisture for setting are advised to undergo testing using gypsum moulds [20]. Therefore identical gypsum cylinders with standardized simulated root canals were chosen. In contrast to natural teeth, where the morphology of root canals can vary, these models provided an opportunity to standardize the amount of RCS in the samples.

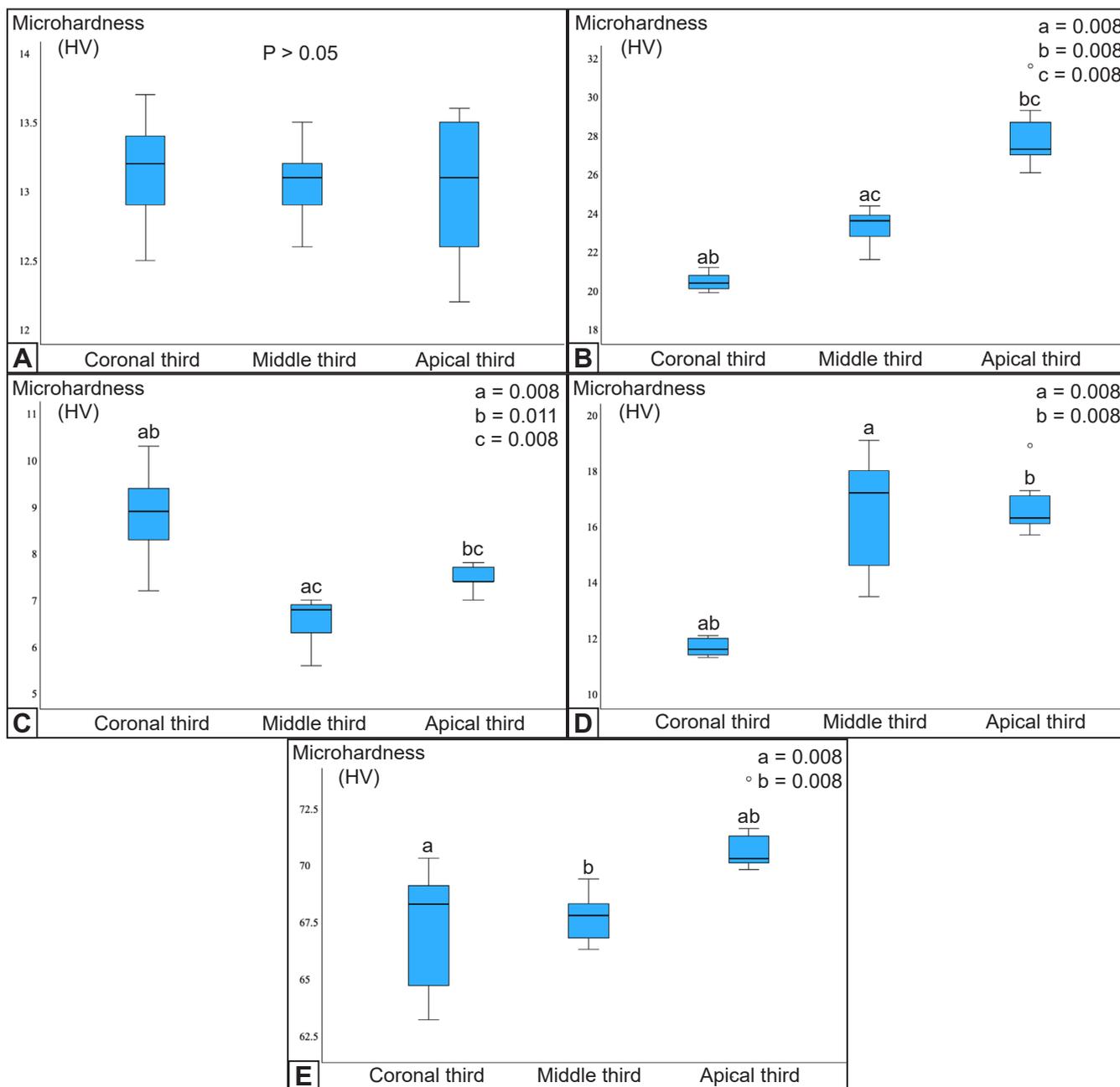


Figure 3. Microhardness of the tested root canal sealers in the coronal, middle and apical thirds: A = TotalFill® BC Sealer™; B = AH Plus® Bioceramic Sealer; C = BioRoot™ RCS; D = AH Plus® Jet™; E = ProRoot® MTA.

Different letters (a to c) indicate statistically significant differences between thirds (P < 0.05, Wilcoxon signed-rank test).

Table 2. Comparison of the microhardness (HV) of the tested materials

Material	Coronal third		Middle third		Apical third	
	Median (IQR)	P-value	Median (IQR)	P-value	Median (IQR)	P-value
TotalFill® BC Sealer™	13.2 (2.85; 13.45) ^{a,b}	0.146	13.1 (12.85; 13.3) ^{a,b}	0.146	13.1 (12.6; 13.55) ^{a,b}	0.146
AH Plus® Bioceramic Sealer	8.9 (8.15; 9.65) ^c	0.146	6.8 (5.95; 6.9) ^c	0.146	7.4 (7.25; 7.75) ^c	0.146
BioRoot™ RCS	20.4 (20.05; 20.9) ^{a,d}	0.146	23.6 (22.35; 23.95) ^{a,d}	0.146	27.3 (26.7; 29) ^{a,d}	0.146
AH Plus® Jet™	11.6 (1.4; 12.05) ^{b,c}	0.146	17.2 (14.15; 18.15) ^{b,c}	0.146	16.3 (16; 17.2) ^{b,c}	0.146
ProRoot® MTA	68.3 (64.15; 69.3) ^d	0.146	67.8 (66.65; 68.7) ^d	0.146	70.3 (70.05; 71.45) ^d	0.146

Superscript letters (a to d) indicate statistically significant differences between materials within each third of the root canal, according to the compact letter display (CLD) method. Materials that share the same letter are not significantly different, whereas materials that do not share any letters are significantly different (P < 0.05, based on the non-parametric Kruskal-Wallis test followed by Dunn’s post-hoc test). IQR = interquartile range.

The use of identical gypsum models allowed for standardization of sample geometry and volume, reducing variability typically associated with natural teeth. Furthermore, the use of gypsum material resulted in the establishment of optimal conditions for the regulation and preservation of the required moisture levels within the root canals.

As demonstrated in other studies, the Vickers microhardness test is the most common tool utilized to determine microhardness [12,17]. This methodology was chosen based on its frequent application in similar studies investigating the mechanical properties of endodontic sealers, particularly bioceramic materials. Vickers microhardness testing has been widely used to assess the surface hardness and setting behaviour of calcium silicate-based sealers and other root canal filling materials, providing reproducible and comparable results [12,17,21]. Surface microhardness allows the assessing of material hydration during the setting reactions [22]. This indicates how effectively water participates in the chemical processes during the material's setting. Previous research results suggest that both environmental conditions and the time elapsed since the sealer's mixing can affect the microhardness of bioceramic RCS [6,12]. It has been established that different environments, particularly those of an acidic nature, can diminish the microhardness of these materials [12]. It is important to note that, in this study, efforts were made to simulate physiological conditions by incubating the samples at 37 °C and 95% humidity. However, real clinical situations may vary depending on the individual case. It is established that inflamed tissues tend to have an acidic pH (pH ≈ 5.5) [23], whereas healthy pulp blood is slightly alkaline (pH ≈ 7.4) [24]. Thus, the microhardness of RCS may vary in clinical practice.

ProRoot® MTA was selected as the positive control due to its status as the most extensively studied bioceramic material. It is known for its clinical effectiveness and is frequently employed in comparative analyses with other RCS [25]. Some studies have demonstrated that ProRoot® MTA exhibits higher microhardness in comparison to other calcium silicate-based cements [26]. AH Plus® Jet™, an epoxy resin-based RCS, is also widely recognised as one of the standard materials in endodontic studies. In the majority of studies examining the properties of bioceramic RCS, it is utilised as a reference material [27] due to its well-known and stable physical and chemical characteristics [5].

In this study, the microhardness values of TotalFill® BC Sealer™, BioRoot™ RCS and AH Plus® Jet™ differed from those reported by other authors

[12,28]. The bioceramic RCS demonstrated greater microhardness, while the resin-based RCS demonstrated reduced microhardness. This discrepancy may be attributed to the use of polyethylene moulds in the studies by Yang et al. [12] and Kapralos et al. [28], which may have affected the hydration of bioceramic samples but created more favourable conditions for the setting of the hydrophobic AH Plus® Jet™. Furthermore, the microhardness of AH Plus® Jet™ in our study ranged from 11.6 HV (in the coronal third) to 17.2 HV (in the middle third), which is significantly higher compared to the 4.9 HV reported after 24 hours in the study by Kim et al. [21].

No studies were found in the literature comparing the microhardness of RCS in different thirds of the root canal. Despite the evidence that bioceramic RCS achieve adequate surface hardness [12,28], their moisture-dependent setting raises concerns regarding whether sufficient humidity is present in the deeper parts of the canal for proper material hardening. In the present study, all tested bioceramic RCS demonstrated either stable (TotalFill® BC Sealer™) or even higher hardness (BioRoot™ RCS) in the apical regions compared to the coronal third, with the exception of AH Plus® Bioceramic Sealer. Therefore, regardless of the sealer type, whether it is a pre-mixed material (TotalFill® BC Sealer™) or mixed prior to application (BioRoot™ RCS), the moisture level in the canal was sufficient to ensure proper setting.

Conversely, the microhardness of AH Plus® Bioceramic Sealer was highest in the coronal third and the lowest overall when compared to the other tested materials. This finding is consistent with other studies, which have reported that AH Plus® Bioceramic Sealer exhibits inferior physical properties compared to other bioceramic endodontic sealers [29]. This may be due to the fact that AH Plus® Bioceramic Sealer contains only 5 to 15% calcium silicates [30], which is significantly less than in the other bioceramic RCS evaluated.

Despite the significant findings, it is imperative to acknowledge the limitations of this study. Due to the research being conducted under *in vitro* conditions, the results obtained may not fully reflect the actual clinical environment. *In vivo* conditions are subject to the influence of external factors, including the composition of biological fluids, mechanical stress and individual tissue characteristics, which may affect the behaviour of the materials. Furthermore, the utilisation of standardized canal models limits the ability to assess the performance of RCS in root canals with varying morphologies. Randomization was not applied, although all specimens were identical, which may limit generalizability.

Only a single time-point (24 hours post-obturation) and a single environmental condition (37 °C and 95 % humidity) were evaluated, preventing observation of long-term or environment-dependent changes. Additionally, pre-hydration was applied only to bioceramic sealers, as required for proper setting and to mimic procedures in the clinical setting, which may influence microhardness outcomes. For group comparisons, the Kruskal-Wallis test followed by Dunn's post-hoc test was used, and the Wilcoxon signed-rank test was applied for paired comparisons. As this investigation was a pilot study intended to inform and guide future research with larger sample sizes, extended observation periods, and more clinically relevant conditions, no multiple comparison correction was applied, which may increase the risk of type I errors.

CONCLUSIONS

The microhardness of root canal sealers exhibited variability across different canal thirds. TotalFill® BC Sealer™ demonstrated stability, BioRoot™ RCS exhibited characteristics analogous to the positive control and the remaining sealers exhibited region-dependent differences in hardness.

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