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Comparative Evaluation of the Sealing Ability of a BioCeramic Sealer (iRoot SP) with AH Plus Sealer with Root Canal Dentin Using Three Different Techniques of Sealer Application: A Combined Dye Extraction and Scanning Electron Microscope Study

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Abstract: The adaptation of a sealer along with the periphery of the dentinal tubules of the root canal is studied. Various techniques have been used for the application of these sealers onto the canal wall for better adaptation but have not been compared to date. The purpose of the study was to comparatively evaluate the sealing ability of a bioceramic sealer with AH plus sealer with root canal dentin using three different techniques for the application of sealer. One hundred twenty extracted maxillary and mandibular anterior teeth were collected, disinfected, and decoronated at the cemento-enamel junction to maintain a standard working length for all samples. The establishment of the working length (40.10) and instrumentation was performed using a rotary instrument, along with a standard irrigation regimen. The teeth were then divided into two main groups according to the sealer used, i.e., Group A (AH Plus) and Group B (iRoot SP). These two main groups were categorized into three sub-groups depending on the technique of sealer placement, i.e., Subgroup 1 (master cone gutta-percha), Subgroup 2 (bidirectional spiral), and Subgroup 3 (passive ultrasonic activation). Out of the 20 samples, 15 samples were randomly allocated for the assessment of sealing ability using the routine dye extraction method, and to verify the results of the dye extraction method, a more advanced evaluation method, i.e., SEM evaluation, was utilized further. To this end, five random samples from each subgroup were allocated for SEM analysis. The obtained scores were then statistically analyzed using an ANOVA test and Post Hoc Tukey's test. In the current study, statistical significance was seen among the three main groups and six subgroups with p-values < 0.005. Subgroup B3 performed significantly better than the other subgroups in both the dye extraction method as well as in SEM analysis. The highest microleakage was shown by subgroup A1; it also exhibited poor penetration of sealer in SEM evaluation. The bioceramic sealer (iRoot SP), when applied using passive ultrasonic activation, showed the best results in both the dye extraction method and the SEM evaluation.

Keywords: AH Plus sealer; bioceramic sealer; bidirectional spiral; dye extraction method; iRoot SP; SEM; ultrasonic activation

1. Introduction

After the completion of the chemo-mechanical preparation of root canals, the complete sealing of the root canal system plays a vital role in the determination of the long-term success of endodontic treatment. Minimizing the leakage and achieving a fluid-tight seal are important objectives of root canal obturation [1]. A completely sealed root canal system prevents oral pathogens from colonizing and re-infecting the root and periapical tissues [2]. Endodontic failures are due to one of the most significant causes, i.e., microleakage, which takes place owing to any compromised contact between the sealer and dentin or the gutta-percha and sealer or to voids accumulating inside the sealer [3,4].

While obturating the root canal, gutta-percha (GP) is used along with a root canal sealer to improve the homogeneity of root fillings because gutta-percha does not adhere to the dentinal walls; hence, the sealer must fill in the irregularities. The root canal sealers seal not only the entire length of the canal but also the apical-foramen, canal irregularities, and any minor irregularities present between the core material and root canal dentin [5].

AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) is one of these resin-based sealers, whose physicochemical properties have been extensively evaluated along with its biological reaction and interfacial adaptation to the root canal dentin. Launched in 2009, EndoSequence® BC (Brasseler, Savannah, GA, USA) is a bioceramic root canal sealer, also known as iRoot SP® (Innovative Bioceramix, Vancouver, BC, Canada), that was introduced in the clinical scenario as an ideal premixed and injectable biomaterial with excellent radiopacity, zero shrinkage, insolubility, and hydrophilic characteristics (using dentinal tubule moisture to initiate and complete its setting reaction). EndoSequence BC/iRoot SP allows the presence of water for it to set and harden, unlike other bioceramic root canal sealers [6].

Researchers also found that the sealers were unable to enter the accessory canals with conventional sealer placement techniques, i.e., using master cone gutta-percha, lentulospirals, etc. [7]. The invention of the bidirectional spiral is a recent advancement in the sealer placement technique. This consists of coronal grooved spirals traveling in the apical direction and apical reverse spirals traveling in a coronal direction, bearing the cement coronally at the point of collision, causing the cement to be forced to travel laterally through the walls, lateral canals, and any other potential invaginations [5]. Musikant, Cohen, and Deutsch found that the bidirectional spiral coated the canal and avoided the apical exit of excess cement [7].

Richmann first applied ultrasonic instruments in endodontics in 1957 [8]. A greater agitation of ultrasound-promoted irrigating solutions intensifies penetration in a region of anatomical complexity, such as the dentinal tubules, and thus enhances the capacity to clean [8]. The activation of root canal sealers can favor the penetration of root canal sealers within the dentinal tubules, resulting in increased sealing and antimicrobial effects. The implications of the sealer's ultrasonic activation into the root canal and the filling consistency have not been thoroughly explored [9].

Therefore, the purpose of this study was to comparatively evaluate the sealing Ability of a bioceramic sealer (iRoot SP) with AH Plus sealer with root canal dentin using three different techniques of sealer application using combined dye extraction and scanning electron microscope technique. The null hypothesis stated that there would be no difference in the sealing ability of bioceramic sealer (iRoot SP) and AH Plus sealer with root canal dentin using three different techniques: master cone gutta-percha, bidirectional spiral, and ultrasonic activation of sealer application when evaluated using the dye extraction method and scanning electron microscope.

2. Materials and Methods

This investigation was conducted in accordance with the Declaration of Helsinki (2013) and approved by the Institutional Ethics committee (approval number SVIEC/ON/DENT/BNPGZ81; date of approval: 16 August 2019). This study followed the CRIS guidelines for in vitro studies. In this study, single-rooted, human maxillary, and mandibular anterior teeth extracted for periodontal and/or orthodontic reasons were collected (Figures 1 and 2).

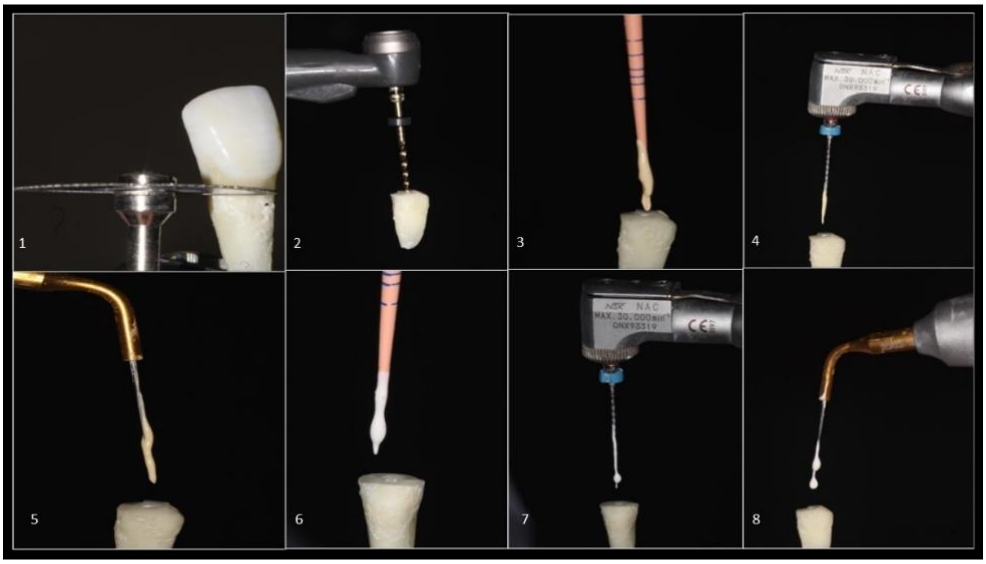

Figure 1. (**1**). Decoronation using a straight handpiece (**2**). Canal prepared using rotary files (**3**). AH plus sealer application using master cone (A1) (**4**). AH plus sealer application using bi-directional (A2) (**5**). AH plus sealer application using ultrasonic file (A3) (**6**). iRoot SP sealer application using master cone (B1) (**7**). iRoot SP sealer application using bi-directional (B2) (**8**). iRoot SP sealer application using ultrasonic file (B3).

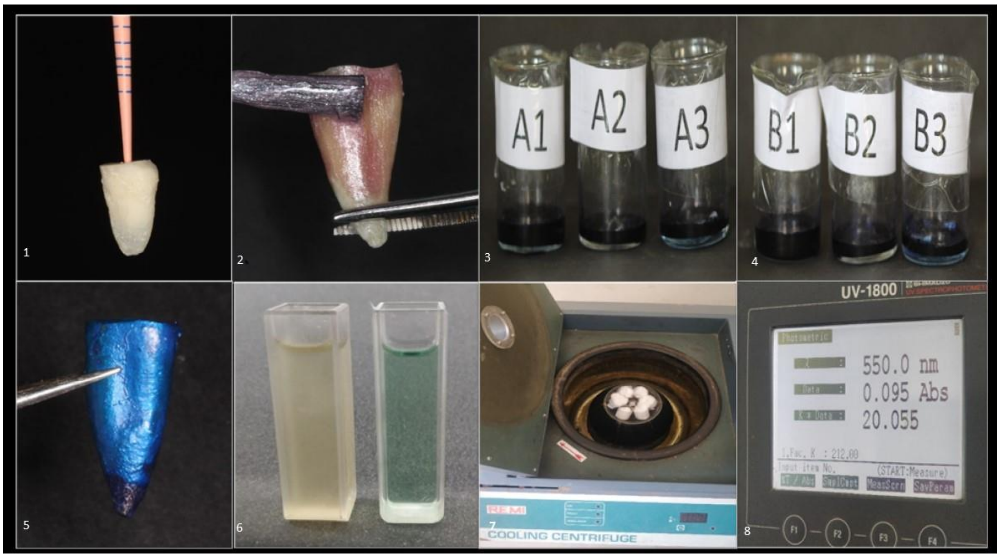

Figure 2. (**1**). Single cone obturation using gutta-percha master cone (**2**). Nail varnish application (**3**). Samples underwent segregation and stored in methylene blue for 24 h (**4**). Samples underwent segregation and stored in methylene blue for 24 h. (**5**). Samples removed from methylene blue. (**6**). Centrifuged at 5000 rpm for 15 min (**7**). 200 microliters of supernatant solution in cubettes (**8**). Absorbance values for each sample recorded using a spectrophotometer.

The sample size was calculated according to the study design by Huang et al. The sample size was calculated using the following formula: $(\text{Zalpha} + \text{Zbeta})^2 * \text{Sqrt}(\text{n*delta}^2/2\text{kS}^2)$. The total sample of 120 subjects, 20 per group, was achieved with 80% power to detect differences among the means versus the alternative of equal means using an F test with a 0.05000 significance level. All teeth were decontaminated in Chloramine-T 0.5% for 24 h and would thereafter undergo thorough scaling and storage in the saline solution until required. The decoronation of teeth was undertaken at the tooth's CEJ with the help of a diamond disk, which was water-cooled to maintain the standard working length of all the samples. (Figure 1(1)), followed by the insertion of a no.10 stainless-steel K file until its tip was visible at the apex. The subtraction of 0.5 mm from this obtained length gave the desired working length. The teeth were then stored in normal saline (0.9% NaCl + H_2O) (Otsoka Pharmaceuticals, India Pvt. Ltd., Ahmedabad, India) until needed. After confirming the presence of a single canal with IOPA radiographs, the instrumentation of all specimens to the size of 40/06% rotary files (Neoendo X7 series, Orikam Healthcare India Pvt. Ltd., Gurugram, India) was completed (Figure 1(2)). Irrigation was performed using 5 mL of 5.25% NaOCl (Vishal Dentocare Pvt. Ltd., Ahmedabad, India) between each instrument with a final rinse of 5 mL of 17% EDTA (Prima Dental Products Pvt. Ltd., New Delhi, India) for 1 min, followed by copious amounts of distilled water (sterile). Samples were taken randomly and divided into 2 main groups according to the sealer used, and each main group was divided further into 3 subgroups (n = 20) according to the technique of sealer placement.

The teeth were divided into two experimental groups:

Group A: AH Plus sealer (Dentsply Maillefer, Ballaigues, Switzerland) (n = 60)
Group B: Bioceramic sealer (iRoot SP, Innovative Bioceramix Inc., Vancouver, BC, Canada) (n = 60)

Group A and Group B were further divided into 3 subgroups each according to the sealer application: Group A: AH Plus sealer (n = 60)

Subgroup A1: AH Plus sealer application using gutta-percha master cone (n = 20)
Subgroup A2: AH Plus sealer application using bidirectional spiral (n = 20)
Subgroup A3: AH Plus sealer application using passive ultrasonic activation (n = 20)
Group B: iRoot SP (n = 60)
Subgroup B1: iRoot SP sealer application using gutta-percha master cone (n = 20)
Subgroup B2: iRoot SP sealer application using bidirectional Spiral (n = 20)
Subgroup B3: iRoot SP sealer application using passive Ultrasonic Activation (n = 20)

Drying the canal

Usually, the canals are dried with the help of sterile paper points right before obturation, but here the protocol was slightly different. As both the sealers required different conditions inside the canal, the required considerations were given following the instructions per the manufacturers before applying them inside the canal. Since iRoot SP is a bioceramic sealer, the canals of Group B were not completely dried; some amount of moisture was left inside the canal to facilitate its setting, simulating the clinical conditions. This is why, instead of using paper points, syringe aspiration was performed. Aspirating the moisture from the canal with the help of the syringe did not render the canals dry. To ensure the wetness of the canal walls, the samples were visualized under a 1.6X magnification factor with 10X actual magnification (Labomed Prima; Labomed India Pvt Ltd., Gurgaon, India).

Subgroup A1 and B1: Gutta-percha master cone (Dental Avenue India Pvt. Ltd., Mumbai, India) (n = 20) (Figure 1(3,6).

Subgroup A1: After drying the canal with sterile paper points, a master gutta-percha cone size/taper 40/06 (Dental Avenue India Pvt. Ltd., Mumbai, India) taper was selected, and its fit was confirmed by taking a radiograph. The sealer was placed inside the root canals using a master cone, moving the master cone for 20 s using a 'hydraulic motion'.

Subgroup B1: Keeping the canals moist, aspirating the moisture from the canal with the help of the syringe, but not completely drying it, the iRoot SP sealer was placed in the canal. After applying in the canal, the sealer was moved in all directions with the help of the master gutta-percha cone using a 'hydraulic motion' for 20 s. This motion helps the bioceramic sealer to be adapted properly on all the sides of the canal walls of the root canals; it also removes the vapor-lock effect in the apical zone.

Root canals were then obturated with the cold lateral compaction technique.

Subgroups A2 and B2: Bidirectional spiral (EZ Fill Spread; Essential Dental Systems, Hackensack, NJ, USA) (n = 20) (Figure 1(4,7))

The respective sealers were placed inside the root canals using a bidirectional spiral mounted on a micromotor handpiece at speed level 3 for 20 s each in buccolingual and mesio-distal directions. Root canals were obturated with a gutta-percha master cone size/taper 40/06 (Dental Avenue India Pvt. Ltd., Mumbai, India) using the cold lateral compaction technique.

Subgroup A3 and B3: Passive ultrasonic activation (n = 20) (Figure 1(5,8))

The ultrasonic tip was coated with the respective manipulated sealer and carried into the prepared root canal. The passive ultrasonic activation of the sealer in the prepared root canal was undertaken for 20 s in the buccolingual direction and 20 s in the mesiodistal direction, keeping it 2 mm away from WL with the insertion of a size 20.02% taper ultrasonic device (Satelec, France) at power level 1 as a standardization procedure.

The root canal was obturated with a gutta-percha master cone size/taper 40/06 (Dental Avenue India Pvt. Ltd., Mumbai, India) using the cold lateral compaction technique (Figure 2(1)). This technique involves placing a single cone of gutta-percha (GP) with sealer in the prepared root canal and adding secondary GP cones that are compacted together with the use of a spreader. The cones stay together because of the frictional grip and the presence of a sealer. After the obturation, the samples were kept under observation for 24 h to allow both sealers to set completely because both the sealers have setting times well within the range of 10 h, 8 h for AH plus and 9.5 h for iRoot SP, per the manufacturer's instructions. The materials used in the experimental study are summarized in Table 1.

Out of the 20 samples, 5 samples from each group were randomly allocated for SEM analysis, and the remaining 15 samples were used for the assessment of sealing ability by the dye extraction method.

Table 1. Material used in the study.

Product Name	Manufacturer	Components	Batch	Expiry
Chloramine-T	Sigma Aldrich Co. LLC	N-chloro-p-toluene Sulfonamides Sodium salt	0269900500	11/2021
Saline	Otsoka Pharmaceuticals India Pvt.Ltd	100mL container Sodium salt IP Water	204958	07/2023
Sodium hypochlorite	Vishal Dentocare Pvt. Ltd.	Sodium hypochlorite solution 5.25% w/w	VM-04	Pack-1/6/20 1/6/22
EDTA	Prima Dental Products Pvt. Ltd.	Carbamide peroxide Ethylenediamine Tetra acetic acids	1912-04	11/2021
K-files	Mani	Stainless steel	0483	
Rotary files	Orikam Healthcare India Pvt. Ltd.	Nickel-titanium	9102008010	
AH Plus sealer	Dentsply Pvt. Ltd.	1 tube paste A 1 tube paste B 1 mixing pad	1808000105	31 July 2021
iRoot SP	Innovative Bioceramix Inc. Canada	PerloaPreloadedge 1 mL (2 gm)	19003SP	30 September 2021

Table 1. *Cont.*

Product Name	Manufacturer	Components	Batch	Expiry
Gutta-percha cones	Dental Avenue India Pvt. Ltd.	Gutta-percha Zinc oxide Barium sulfate Stearic acid	010617	09/2022
Paper points	Diadent Group International (Korea)		011018	09/2022
EZ Fill spread	Essential Dental Systems, USA	Stainless steel bi-dl SP	1600-21	
Nitric acid	Aatur Instru Chem Vadodara	Arsenic Chloride/hydrochloric acid		

2.1. Microleakage Evaluation

1. A total of 15 samples from each subgroup of 20 were allocated by computerized randomization.

Prior to the assessment of microleakage, all tooth surfaces were coated with two coats of nail varnish, except for a 3 mm portion around the apical foramen (Figure 2(2)).

2. The apices of the roots were dipped for 24 h at 37 °C in a neutralized buffer 2% methylene blue solution under normal atmospheric pressure.

3. Thereafter, the teeth were removed from the dye, and superficial dye was removed with pumice slurry.

4. Varnish was removed using a BP blade and polishing disks. Then, they were transferred to a sterile container containing 6 mL of 65% nitric acid for 6 days.

5. A dilution was made using 0.15 mL of acid solution from the specimen and 1.35 mL of distilled water.

6. The centrifugation of this solution was performed at 14,000 rpm for 3 min to separate debris from the extracted dye.

7. Next, 1 mL of supernatant was transferred to the measuring cubes of the spectrophotometer (Shimadzu UV 1800) using micro-pipettes (Figure 2(7)).

8. The absorbance of each sample was determined using an automatic absorbance UVVIS spectrophotometer (Shimadzu UV 1800) at 550 nm, taking concentrated nitric acid as a blank (Figure 2(8)).

2.2. Sealer Adaptation Evaluation

- For the purpose of the scanning electron microscope evaluation, five random samples from every group were included.
- Roots were sectioned with the help of a diamond bur and a chisel longitudinally in the labiolingual direction and were further sectioned horizontally at 2 mm, 4 mm, and 6 mm from the apical foramen. Sections were then evaluated under an SEM.
- To assess the interfacial adaptation of the sealer, dentinal tubule areas covered by the sealer to the root canal dentin were examined from the coronal third to the apical third at 1500× magnification for the evaluation of the continuity of the sealer, and finally, the microphotographs were recorded.

2.3. Statistical Analysis

Obtained scores were then statistically analyzed using an ANOVA test and Post Hoc Tukey's test, and p-values < 0.05 were considered statistically significant.

3. Results

There was a statistically significant difference between both the groups (Table 2), as well among all six subgroups (Table 3), as the SEM analyses revealed that the adaptation to dentin was sufficient and more homogenous for the bioceramic (iRoot SP) sealer used along with passive ultrasonic activation (Subgroup B3) compared to all other subgroups. The least sufficient was the AH Plus sealer applied using master cone gutta-percha. When compared according to the location from the apex, the coronal sections showed superior sealing where the texture of the sealers in the tubules was homogeneous (Figures 3–5 and Table 4).

Table 2. One-way analysis of variance (ANOVA).

Name of Variables	Name of Grouping Variables	Mean ± SD (Absorbance Values in Abs)	95% CI	*p*-Value
Group A AH Plus sealer	Subgroup A1 Gutta-percha master cone	0.095 ± 0.0091	(0.0903, 0.1004)	<0.001 (<0.05)
	Subgroup A2 Bidirectional spiral	0.056 ± 0.0151	(0.0480, 0.0648)	
	Subgroup A3 Ultrasonic activation	0.086 ± 0.0069	(0.0827, 0.0903)	
Group B bioceramic sealer	Subgroup B1 Gutta-percha master cone	0.082 ± 0.0109	(0.0761, 0.0882)	<0.001 (<0.05)
	Subgroup B2 Bidirectional spiral	0.039 ± 0.0043	(0.0375, 0.0423)	
	Subgroup B3 Ultrasonic activation	0.022 ± 0.0075	(0.0174, 0.0257)	

Table 3. Post Hoc Tukey's tests for multiple comparisons.

Group A (AH Plus Sealer)	Mean ± SD (ABSORBANCE Values in Abs)	Mean Difference	*p*-Value
Gutta-percha master cone vs. bidirectional spiral	(0.082 ± 0.0109) vs. (0.039 ± 0.0043)	0.0423	<0.001 (<0.05)
Gutta-percha master cone vs. ultrasonic activation	(0.082 ± 0.0109) vs. (0.022 ± 0.0075)	0.0606	<0.001 (<0.05)
Bidirectional spiral vs. ultrasonic activation	(0.039 ± 0.0043) vs. (0.022 ± 0.0075)	0.0183	<0.001 (<0.05)
Group B (Bioceramic sealer)	Mean ± SD	Mean Difference	*p*-value
Gutta-percha master cone vs. bidirectional Spiral	(0.095 ± 0.0091) vs. (0.056 ± 0.0151)	0.0390	<0.001 (<0.05)
Gutta-percha master cone vs. ultrasonic Activation	(0.095 ± 0.0091) vs. (0.086 ± 0.0069)	0.0087	0.080 (>0.05)
Bidirectional spiral vs. ultrasonic activation	(0.056 ± 0.0151) vs. (0.086 ± 0.0069)	0.0301	<0.001 (<0.05)

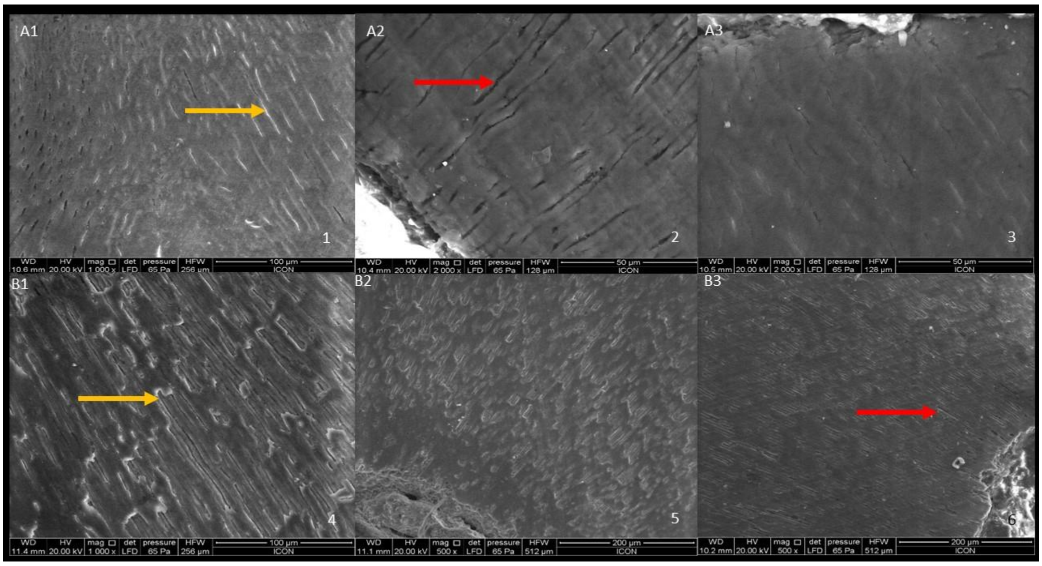

Figure 3. SEM Images of 'Coronal' Sections of all the sub-groups (Yellow arrow indicates the adaptation of the sealer; that is radiopaque, along the length of the tubule, whereas the red arrow indicates the absence or the negligible adaptation of the sealer). (**A1**):-Coronal Section of AH Plus sealer application using gutta-percha master cone. (**A2**):-Coronal Section of AH Plus sealer application using bi-directional Spiral (**A3**):-Coronal Section of AH Plus sealer application using ultrasonic Activation (**B1**):-Coronal Section of iRoot SP sealer application using gutta-percha master cone (**B2**):-Coronal Section of iRoot SP sealer application using bi-directional Spiral (**B3**):-Coronal Section of iRoot SP sealer application using ultrasonic Activation.

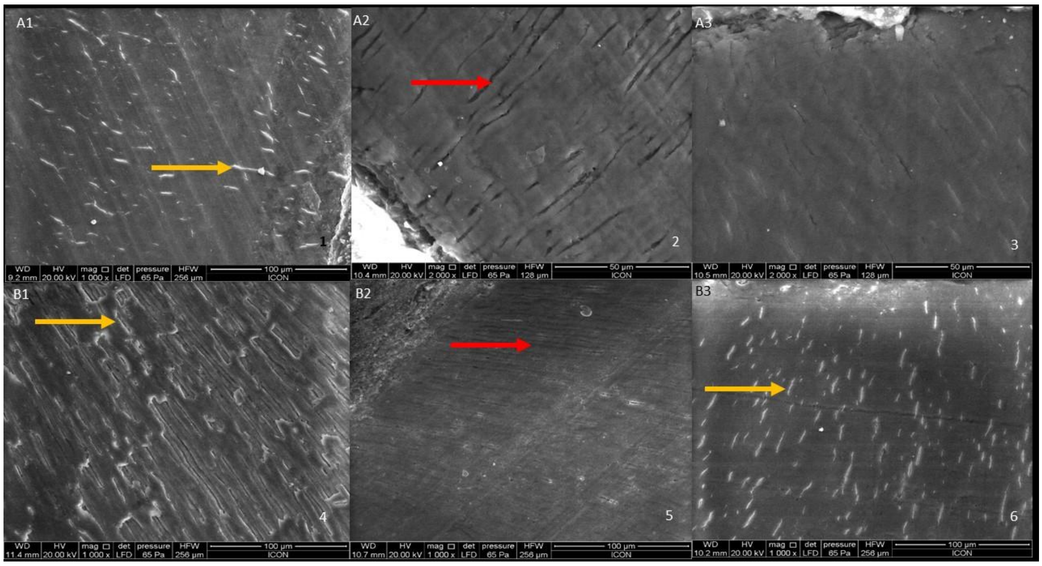

Figure 4. SEM Images of 'Middle' Sections of all the sub-groups (Yellow arrow indicates the adaptation of the sealer; that is radiopaque, along the length of the tubule, whereas the red arrow indicates the absence or the negligible adaptation of the sealer). (**A1**):-Middle Section of AH Plus sealer application using gutta-percha master cone (**A2**):-Middle Section of AH Plus sealer application using bi-directional Spiral (**A3**):-Middle Section of AH Plus sealer application using ultrasonic Activation (**B1**):-Middle Section of iRoot SP sealer application using gutta-percha master cone (**B2**):-Middle Section of iRoot SP sealer application using bi-directional Spiral (**B3**):-Middle Section of iRoot SP sealer application using ultrasonic Activation.

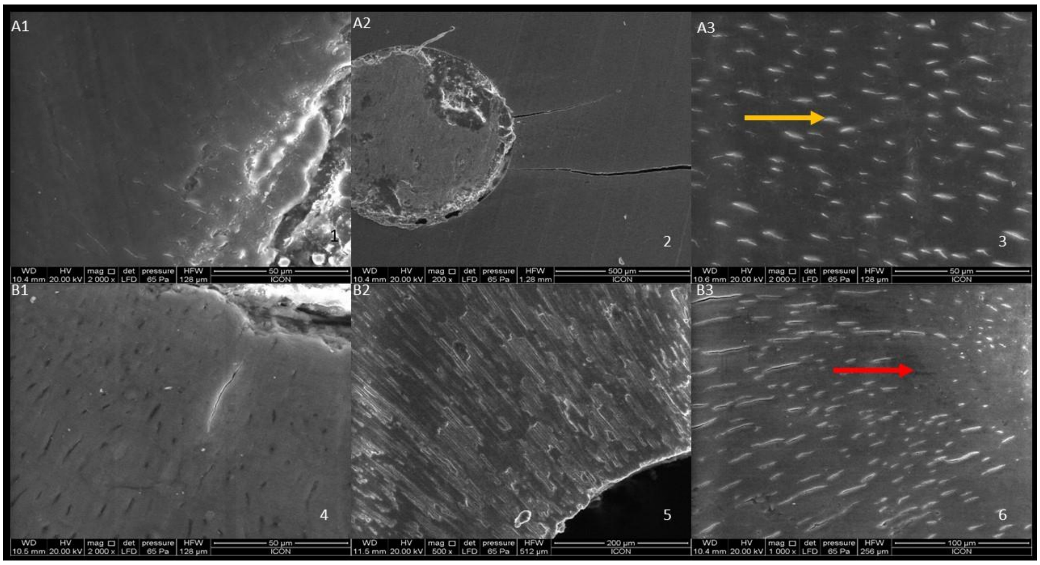

Figure 5. SEM Images of Apicl Sections of All the Subgroups. SEM Images of 'Apical' Sections of all the sub-groups (Yellow arrow indicates the adaptation of the sealer; that is radiopaque, along the length of the tubule, whereas the red arrow indicates the absence or the negligible adaptation of the sealer). (**A1**):-Apical Section of AH Plus sealer application using gutta-percha master cone (**A2**):-Apical Section of AH Plus sealer application using bi-directional Spiral (**A3**):-Apical Section of AH Plus sealer application using ultrasonic Activation (**B1**):-Apical Section of iRoot SP sealer application using gutta-percha master cone (**B2**):-Apical Section of iRoot SP sealer application using bi-directional Spiral (**B3**):-Apical Section of iRoot SP sealer application using ultrasonic Activation.

Table 4. SEM EVALUATION OF Group A and Group B.

				AH Plus		
		1	**2**	**3**	**4**	**5**
	Coronal	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
A1	Middle	Continuous	Discontinuous	Discontinuous	Continuous	Discontinuous
	Apical	Discontinuous	Discontinuous	Discontinuous	Continuous	Discontinuous
	Coronal	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
A2	Middle	Continuous	Continuous	Continuous	Continuous	Continuous
	Apical	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
	Coronal	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
A3	Middle	Continuous	Continuous	Continuous	Continuous	Continuous
	Apical	Continuous	Continuous	Continuous	Continuous	Continuous
				iRoot SP		
		1	**2**	**3**	**4**	**5**
	Coronal	Continuous	Continuous	Continuous	Continuous	Continuous
B1	Middle	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
	Apical	Discontinuous	Discontinuous	Discontinuous	Continuous	Discontinuous
	Coronal	Continuous	Continuous	Continuous	Continuous	Continuous
B2	Middle	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Discontinuous
	Apical	Continuous	Continuous	Continuous	Continuous	Continuous
	Coronal	Continuous	Continuous	Continuous	Continuous	Continuous
B3	Middle	Continuous	Continuous	Continuous	Continuous	Continuous
	Apical	Continuous	Continuous	Continuous	Continuous	Continuous

The statistical test applied for the comparison of means of samples when they are three or more in number is the ANOVA. Data that are numerical in nature are used for the application of the ANOVA test. TheANOVA statistical test for this study revealed results in which the absorbance value for Subgroup A1 (0.095) is the highest, followed by Subgroup

A3 (0.086), Subgroup B1 (0.082), Subgroup A2 (0.056), and Subgroup B2 (0.039), and the least was Group B3 (0.022). The difference obtained has statistically significant importance (p-value is < 0.001) (Table 2).

For multiple comparisons test, Tukey's HSD Post Hoc Test was used in this study wherein the difference between Subgroup A1 and Subgroup A2 is statistically significant with a mean difference equaling to a p-value < 0.01. The difference between Subgroup A1 and Subgroup A3 is not of statistically significant importance, with a mean difference equaling to a p-value of 0.08. The difference obtained between Subgroup A2 and Subgroup A3 is of statistically significant importance, with a mean difference equaling a p-value of <0.01. The difference obtained between Subgroup B1 and Subgroup B2 is of statistically significant importance, with a mean difference equaling to a p-value of < 0.01. The difference obtained between Subgroup B1 and Subgroup B3 is of statistically significant importance, with a mean difference equaling to a p-value of <0.01. The difference between Subgroup B2 and Subgroup B3 is of statistically significant importance, with a mean difference equaling to a p-value of < 0.01 (Table 3).

4. Discussion

Hermetic sealing is the primary factor associated with the success of root canal treatment, and Ingle et al. (2008) [10] pointed out that 58% of treatment failures were due to incomplete obturation. As AH Plus is considered a gold standard root canal sealer according to previously stated literature, we decided to compare this sealer to the recently introduced bioactive iRoot SP sealer [11].

In the past, only a few studies were conducted on the effect of sealer activation/placement on sealing ability, depth of penetration, and interfacial adaptation of root canal sealers [12–16]. Therefore, in this present study, three different techniques of sealer activation/agitation were chosen, and the sealer distribution was analyzed using the dye extraction method and SEM evaluation.

Out of several microscopy techniques available, SEM offers advantages like the use of non-decalcified or hard tissue samples not requiring specific sputter coating. It also provides detailed information about the presence and distribution of sealers at variable magnification and allows the exclusion of artifacts from the sample [17,18].

In our study, in the results of the dye extraction method for Group A, i.e., the AH Plus sealer group, the least microleakage was shown by Subgroup A3, in which AH Plus was placed by passive ultrasonic activation. Maximum microleakage for Group A was shown by Subgroup A1. The dye extraction method results for Group B, i.e., iRoot SP, revealed the least absorbance values for Subgroup B3, i.e., iRoot SP applied by passive ultrasonic activation. However, the highest microleakage was shown by Subgroup B1 (Table 2).

Upon comparing Group A and Group B, owing to mean absorbance values of microleakage, Subgroup A1 showed the highest mean (microleakage), followed by Subgroup A3, Subgroup B1, Subgroup A2, and Subgroup B2, and the least microleakage was shown by Subgroup B3.

This may be attributed to passive ultrasonic activation favoring a greater dentinal sealer penetration and improving the interfacial adaptation between the sealer and the root canal walls [19]. Similarly, using a fluid filtration method and SEM, Zhang et al. (2009) investigated the sealing ability of the iRoot SP sealer and AH Plus sealer to the apical section of teeth roots. It was found that the iRoot SP using the single-cone technique and the AH Plus using the continuous-wave condensation technique were equivalent in fluid leakage. SEM also revealed that both sealers provided gap-free and gap-containing regions within the canals [20]. Contradictory to these findings, the SEM observation in the present study showed that the apical adaptation of the iRoot SP was better than that of the AH Plus sealer [21]. The possible reason for this could be the passive ultrasonic activation. This is in accordance with the study conducted by De Bem et al. [22], where passive ultrasonic activation significantly enhanced the dentin tubule penetration of all the sealers, including a bioceramic sealer. This could be related to the results of the study conducted by

Wang et al. [23], which showed that iRoot SP performed better than AH plus in terms of sealer penetration, especially in the apical zone. Another important reason for better adaptation for Subgroup B3 (iRoot SP with passive ultrasonic activation) could be the particle size of sealer iRoot SP of 2 μm [24], which is almost equal to the dentinal tubules in the apical third [25], which was 1.73 μm on average. It has also been confirmed that iRoot SP could penetrate into the dentinal tubules, which have diameters ranging from 1 to 3 μm [23]. Therefore, the inherent property of the small particle size of iRoot SP combined with the passive ultrasonic activation helped Subgroup B3 perform better in the apical third.

In the SEM analysis for Group A, i.e., the AH Plus sealer group, Subgroup A1 showed the incomplete, inconsistent, and discontinuous distribution of the sealer in all three sections of the teeth, i.e., coronal, middle, and apical third. For Subgroup A2, of the three sections, only the middle third showed continuous and homogenous sealer distribution into dentinal tubules. The coronal and middle third sections were discontinuous in their sealer distribution. Maximum penetration for the AH Plus sealer group was seen only with Group A3. Unlike the coronal third, which showed discontinuous sealer distribution, both the middle and apical thirds showed continuous filling of the dentinal tubules.

The SEM analysis for Group B, i.e., the iRoot SP group, showed the following results. The coronal sections of all three subgroups showed continuous filling of the dentinal tubules using all three sealer placement techniques. In Subgroup B1, except for its coronal third, there was no continuity in the sealer distribution within the dentinal tubules. It was only for Subgroup B3 that all three sections showed the continuous and homogenous distribution of sealer within the dentinal tubules. This could again be related to the results of the study by Wang et al., which showed that iRoot SP performed better than AH plus in terms of sealer penetration, especially in the apical zone [23]. Another reason, as mentioned previously in the discussion, is that the iRoot SP contains nanoparticles (about 2 μm in diameter), facilitating the penetration into dentinal tubules [23–27]. The null hypothesis stated in the study, hence, was rejected.

The reason for having two different methods of evaluation was to have more clarity on the results. The SEM evaluation was conducted just to have an idea of whether the results of the dye extraction methods were in accordance with the SEM analysis or if there might be any discrepancies between the methods. This is why, even after performing the spectrophotometer analysis, a few samples (n = 5) from every group were tested through the SEM evaluation.

SEM evaluation was conducted just to confirm the adaptation of the sealer to the dentinal tubules. Since a micro-CT analysis was not performed, it was not a volumetric evaluation; neither the measurement of the area of sealer penetration nor the depth was measured. The selected samples from each group were seen under SEM just to check whether the sealer had adapted to the tubules' length. This is why the eventual SEM images were evaluated for two simple observations: 1. continuous and 2. discontinuous. The presence of the sealer along the length of the tubules (radiopacity in the tubules) was termed 'continuous', whereas the negligible amount or absence of sealer (radiolucency in the tubules) was called 'discontinuous'. These evaluations were based on the observation of two separate experienced and calibrated observers who had more than 80 percent agreement on the kappa test.

Since the number of samples evaluated under SEM was lower, a study with a larger number of samples is required to validate the result of the present study. Also, one micro-CT evaluation needs to be conducted to shed more light on this topic.

5. Conclusions

Considering the limitations of this current in vitro research, the following concluding points can be made: iRoot SP (bioceramic sealer) can be the sealer of choice for endodontic treatments in the future along with the application of ultrasonic energy to achieve the most optimal results for root canal treatment with excellent biocompatibility with periapical regions and tissues. Along with SEM, higher evaluation modalities, such as micro-CT, may give further positive support to the finding of the current study. Further research is required comparing bioceramic materials with other materials and different experimental sealer placement studies.

5.1. Clinical Implications

Based on the results of this in vitro study, the clinical implication of the study is that the use of bioceramic sealer along with passive ultrasonic activation gives a better sealer adaptation to the periphery of the dentinal tubules and can further positively impact the quality of the obturation in the clinical scenario.

5.2. Highlights

1. Bioceramic (iRoot SP) sealer showed better penetration in the dentinal tubules than AH plus sealer.
2. Bioceramic (iRoot SP) sealer used along with passive ultrasonic activation showed superior adaptation to the dentinal tubules.
3. The coronal third of the canal showed better sealer penetration regardless of the technique of placement.

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