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Comparative evaluation of dentinal tubule penetration and push-out bond strength of new injectable hydraulic calcium disilicate based root canal sealer: A single blinded in vitro study

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ARTICLE INFO	A B S T R A C T					
A R T I C L E I N F O Keywords: Calcium silicate sealers Confocal laser scanning microscopy Dentinal tubule penetration Push-out bond strength Root canal sealers	<i>Objective</i> : To evaluate and compare dentinal tubule penetration and push-out bond strength of BIO-C ION+, AH Plus and NanoSeal-S using Confocal Laser Scanning Microscopy (CLSM) and Universal Testing Machine (UTM). <i>Materials & method</i> : Sixty human mandibular premolars were prepared using ProTaper Gold till F3. Samples were then divided into 3 groups: Group I ($n = 20$) BIO-C ION + sealer, Group II ($n = 20$) AH Plus and Group III ($n = 20$) NanoSeal-S sealer. Groups were then sub-divided into two sub groups: In Subgroup A ($n = 10$) samples were obturated using single-cone with 0.1 % Rhodamine B dye and in Subgroup B ($n = 10$) samples were obturated using single cone. The samples were then transversely sectioned into coronal, middle and apical segments, samples in subgroup A & B were then submitted to CLSM analysis and UTM respectively. <i>Results</i> : The Bond Strength data showed following means (MPa): Group I Subgroup B: (BIO-C ION+) coronal (1.64), middle (1.25), apical (0.93); Group II Subgroup B: (AH Plus) coronal (2.20), middle (1.85) apical (1.38) and Group III Subgroup B: (NanoSeal-S) coronal (1.26), middle (0.94), apical (0.58). The dentinal tubule penetration data showed following means: (μ m) Group I Subgroup A (BIO-C ION+) coronal (1184.69), middle (997.03), apical (637.26); Group II Subgroup-A AH Plus (864.14) and NanoSeal-S (495.64). Statistical analysis (two-way ANOVA, Tukey's Post Hoc Test) showed significant difference among sealers ($p < 0.001$) and root canal thirds ($p < 0.001$). <i>Conclusion:</i> The results of the study concluded that BIO-C ION + sealer showed maximum dentinal tubule penetration and AH Plus demonstrated maximum push-out bond strength.					

1. Clinical significance

The penetration of root canal sealers into dentinal tubules decreases the interface between the core material and dentin which may increase the retention of the core. The push-out bond strength test yields important details concerning sealers' resistance to occlusal stresses on root canal walls (see Fig. 8–10) (Fig. 8–10).

2. Introduction

An effective root canal therapy involves complete disinfection and 3D sealing of the canals.¹ Gutta percha has always been utilized for obturation. It has various features, including biocompatibility, radiopacity, non-porosity, chemical stability, and manipulability. However,

due to its hydrophobic nature, it lacks adherence to canal walls. To overcome this, an optimal root canal sealer with superior sealing ability is required.Root canal sealers provide a fluid-tight seal and prevent microorganisms from entering the root canal from the oral environment. The aim is to seal the root canal space in three dimensions throughout the whole length preventing leakage, inflammatory exudates, saliva, and chemical substances from entering the canal.¹

Grossman stated that an optimal root canal sealer should have qualities like a great seal after setting, dimensional stability, sufficient adhesion, and biocompatibility. Endodontic sealers can be made of different types of bases, the most recent ones are the calcium-disilicate bioceramic sealers.²Epoxy resin-based sealers are currently the most often utilized and clinically available root canal sealers. Schroeder introduced the AH series prototype in 1957, with exceptional physical

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qualities and sealing capabilities. It has been tested and is usually regarded as the best control to employ in the majority of sealing studies.^{3–6}Davis et al.⁷ in 1972 discovered silicone-based root canal sealers. One such cold-flowable polydimethylsiloxane root canal sealer is NanoSeal-S. Several investigations have found it to be highly biocompatible and effective at preventing bacterial development. However, information on its physical qualities and sealing capacity is limited. ⁸⁻¹⁰Lately, calcium-silicate-based sealers have been created, their sealing capacity is said to be comparable to that of resin-based sealers.¹¹ One such hydraulic Calcium-silicate-based endodontic sealer is BIOC ION+. According to the manufacturers, it has exceptional physical qualities with approximately 67.5 % bioceramic load and flows up to 25 mm.

Sealer penetration is clinically significant. The incorporation of sealer tags into dentin allows for a good adaptation of the sealer cementdentin interface. The good penetration, adaptability, and adhesion qualities have two favorable effects: first, enhanced surface area contact between sealer and dentin,¹² and second, antibacterial impact by trapping residual germs into dentinal tubules.^{13,14} Irrigants, medications, and sealers have been tested for penetration into root dentinal tubules using bleaching, Confocal Laser Scanning Microscopy (CLSM), scanning electron microscopy (SEM), light microscopy and, most recently, radiolabeling.^{15,6}The use of CLSM has advanced and become industry standard since it enables repeatable three-dimensional imaging of the materials without causing any harm to them and tends to produce fewer artefacts.^{17–2}

There have been claims that the push-out test evaluates bonding strength better than the traditional shear test. The bond strength and dislocation resistance of the intraradicular dentine and the filling material were evaluated using thin slices. Using this technique, the binding strength of root canal filling materials has been assessed. Universal Testing Machine (UTM) was used in this study for the assessment of the POBS.As far as we know, no research has previously analyzed and compared the dentinal tubule penetration and push-out bond strength of three distinct compositions of endodontic sealers, even though several root canal sealers have recently been created.

In light of this, the study's objective was to evaluate and compare the dentinal tubule penetration and POBS of three different bases of endodontic sealers which have not been studied to date.

3. Materials and method

The present study was carried out in the Department of Conservative Dentistry and Endodontics at I.T.S Center for Dental Studies and Research Center, Murad Nagar, Ghaziabad, Uttar Pradesh in collaboration with the Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh.

Before the study, the study design was approved by the ethical committee under protocol number ITSCDSR/IIEC/2020-23/CONS/02.

Sixty, single-rooted human mandibular premolar teeth that were recently extracted for orthodontic purposes with intact roots and completely formed apices were chosen for the study.

The inclusion criteria for the teeth will be as follows: Teeth exhibiting only one canal which was confirmed by X-rays with both mesiodistal and bucco-lingual directions, teeth were inspected under a dental operating microscope (10X) to exclude the possibility of any cracks or fractures. Only fully formed roots with closed apices, free from resorption, caries, fractures, dilacerations, and calcifications were selected. Residual soft tissue and the external surfaces of the teeth were cleaned with an ultrasonic scaler. Teeth were immediately stored in a 0.1 % thymol solution (pH 7.0) at 4 $^{\circ}$ C for 24 h.

4. Sample preparation

For standardized length, the crowns were sectioned at 14 mm from the apex with a diamond saw in a slow-speed handpiece under profuse water irrigation. A #10K file was used to check the canal patency. Teeth with canals patent to a size less than #10K or greater than #20K were discarded. The pulp was extirpated using a barbed broach.

5. Biomechanical preparation

#10 K file was placed until the tip was evident at the apex, then 1 mm was subtracted from this length, and the working length was determined and confirmed radiographically. Sticky wax was used to seal the apex. Biomechanical preparation was done using rotary ProTaper Gold (Dentsply-Maillefer, USA) sequentially, up to the F3 instrument, using the crown-down approach. Preparation was carried out with 3 ml of 5.25 % NaOCl in continual irrigation and recapitulation with #10 K- file between each instrument. Glyde File Prep was used for lubrication during instrumentation.

6. Grouping of the samples

The grouping was done according to the sealer used. Samples were allocated at random into three experimental groups (n = 20). These were the groups.

GROUP I (n=20) Obturation done with Calcium–disilicate-based sealer BIO-C ION+.

GROUP II (n=20) Obturation done with Resin-based sealer AH Plus. **GROUP III** (n=20) Obturation done with silicone-based sealer NanoSeal-S.

All the samples of all the groups were further divided into two subgroups (n = 10) according to the parameter evaluated.

SUB-GROUP A (n = 10): Obturation was done using a single cone technique along with 0.1 % Rhodamine dye (CDH, India) to allow CLSM analysis and provisional restoration was given. For 24 h, the samples were kept at 37 °C with 100 % relative humidity.

From each root third, three sections were taken, and 30 slices in total were produced. The samples were polished using silicon carbide sand-paper in decreasing order of granulation after 24 h. The samples were put on a histology slide, mounted with a cover slip, and cleaned in an ultrasonic tank for 10 min before drying at 37 $^{\circ}$ C. F

following single blinding by numbering, CLSM was used to evaluate the samples.

SUB-GROUP B (n = 10): Obturation was done using a single cone technique and provisional restoration was given.

After polishing and cleaning the sections, each section was fastened to a metallic surface that was customized to fit the lower part of the UTM and featured a 2.5-diameter hole in the center. After being singleblinded by numbering, the samples were examined by a Universal Testing Machine.

6.1. Sample evaluation by using a confocal microscope

Nikon (Nikon, U.S.A) Confocal Laser Scanning Microscope was used to analyze all samples (Fig. 1) NIS-Elements AR software (Nikon, U.S.A) was used to analyze the shots (Nikon, U.S.A). The laser was excited at 543 nm. Images from the CLSM were captured in fluorescent mode. Images were taken at X10 with a numeric aperture of 0.4, measuring 1550 \times 1550 mm2, and had a resolution of 512 x 512 pixels A fluorescent ring around the canal wall was looked for in each of the ten samples. The CLSM image recorder's digital measuring ruler was used to take measurements from the resin-dentin junction till the maximum depth in all four directions, and the mean was computed and recorded for each sample.

6.2. Sample evaluation by Universal Testing Machine

Cylindrical pluggers of three different diameters were used (Fig. 2) with a crosshead speed of 0.5 mm/min. POBS was tested by providing a

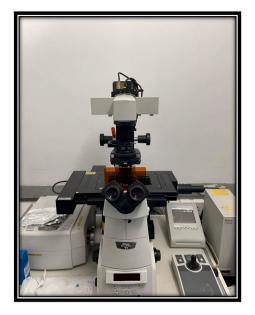


Fig. 1. Confocal laser scanning microscope Nikon (Nikon, U.S.A).

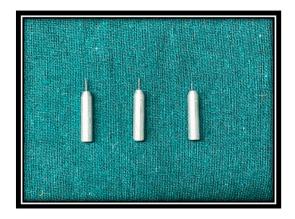


Fig. 2. Plunger Sizes (From left to right: Coronal- 1 mm, Middle-0.50 mm, Apical-0.30 mm).

compressive load to each segment's apical side.

Cylindrical plunger dimensions.

1 mm for coronal specimens 0.50 mm for middle specimens 0.30 mm for apical specimens.

The highest load in Newtons was divided by the area of the bonded interface to calculate the bond strength (MPa). The area of the bonded interface was determined using the formula = 2r X h, where r and h are the measured radius and height in millimeters of the filler material, The Olympus Camedia C-5060 digital camera (Tokyo, Japan) connected to a stereomicroscope (Tokyo, Japan). was used to measure the diameters of the obturated area (Fig. 3).

7. Statistical analysis

7.1. Sample size calculation

With the help of a literature survey, we have found the expected s.d. of the parameter of sub-group 1 & sub-group2 are 1.03 and 1.86 respectively and the mean difference is 1.78 of the two groups for variables. Using the above formula with and software Open Epi, Version 3,

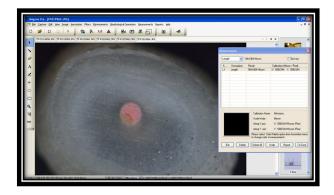


Fig. 3. Determination of radius of obturated area.

we have found the sample size for each group sub group is 10, so in total 60 for three groups. Formula:

$$n = \frac{(\sigma 1^2 + \sigma 2^2) (Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Lambda^2}$$

The notation for the formulae is.

$$\label{eq:sample size of Groups} \begin{split} &n = \text{sample size of Groups} \\ &\sigma 1 = \text{standard deviation of Group } 1 = 5.4 \\ &\sigma 2 = \text{standard deviation of Group } 2 = 4.9 \\ &\Delta = \text{difference in group means} = 4.6 \\ &Z_{1-\alpha/2} = \text{two-sided Z value (e.g., Z = 1.96 for 95 \% confidence interval).} \\ &Z_{1-6} = \text{power} = 80 \% \end{split}$$

The data was analyzed using SPSS software v23. The level of significance was kept at 5 %. Data was subjected to an assessment of normality using the Shapiro-Wilk test. Results of normality testing showed that data was following a normal distribution. Hence, parametric tests were applied.

Comparison of Push-out bond strength and dentinal tubule penetration in each section of the three groups was performed using a Oneway ANOVA test followed by a post hoc Tukey test for pairwise comparisons. Similarly, a comparison of Push-out bond strength and dentinal tubule penetration between three sections of each group was performed using a One-way ANOVA test followed by a post hoc Tukey test for pairwise comparisons.

8. Results

Comparison of dentinal tubule penetration between three sections of each group (Intra-group comparison). One-way ANOVA test; * indicates significant difference at $p \leq 0.05$ Among all the groups, the coronal section showed maximum penetration values followed by the middle and apical sections (Table 1, Graph 1).

Comparison of POBS between three sections of each group (Intragroup comparison). One-way ANOVA test; * indicates a significant difference at $p \leq 0.05$. Among all the groups coronal section showed maximum POBS values with statistically significant differences. (p ≤ 0.05) (Table 2, Graph 2)

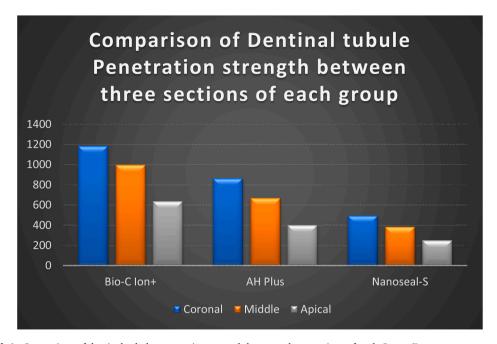
Comparison of dentinal tubule penetration between each section of three groups (Inter-group comparison). One-way ANOVA test; * indicates significant difference at $p \leq 0.05$. At all three sections for all three groups, the BIO-C ION + group showed maximum penetration values followed by AH Plus and NanoSeal-S (Table 3, Graph 3)

Comparison of POBS between each section of three groups (intergroup comparison). One-way ANOVA test; * indicates a significant difference at $p \le 0.05$. At all three sections for all three groups, the AH Plus

Table 1

comparison of dentinal tubule penetration between three sections of each group (intra-group comparison).

Section	Coronal		Middle		Apical		p value
	Mean	SD	Mean	SD	Mean	SD	
Bio-C Ion+ AH Plus Nanoseal-S	1184.69 864.14 495.64	207.09 65.58 100.06	997.03 669.08 384.43	165.92 47.99 71.25	637.26 400.52 250.85	239.79 79.10 77.97	$<\!\!0.001^* \\ <\!\!0.001^* \\ <\!\!0.001^*$



Graph 1. Comparison of dentinal tubule penetration strength between three sections of each Group (Intra-group comparison).

Table 2 comparison of push-out bond strength between three sections of each group (intra-group comparison).

Section	Coronal		Middle		Apical		p value
	Mean	SD	Mean	SD	Mean	SD	
Bio-C Ion+	1.64	0.18	1.25	0.27	0.93	0.15	< 0.001*
AH Plus	2.20	0.23	1.85	0.34	1.38	0.36	< 0.001*
Nanoseal-S	1.26	0.22	0.94	0.15	0.58	0.09	< 0.001*

group showed maximum POBS values followed by BIO-C ION+ and NanoSeal-S (Table 4, Graph 4)

9. Discussion

Root canal therapy attempts to eradicate bacteria and minimize the possibility of re-infection. To achieve the aforementioned goal, a proper fluid-tight seal should be achieved as it reduces the possibility of bacterial proliferation in hard-to-reach areas. Dow and Ingle $(1955)^{18}$ estimated that insufficient or poor root canal sealing was responsible for approximately 60 % of all endodontic failures.

The depth of sealer penetration and dentin bond strength are thus two crucial features to consider when selecting a suitable root canal sealer. According to Tagger et al. (2002),¹⁹ mechanical interlocking causes substrate attachment, and in the case of endodontic sealers, penetration into dentinal tubules determines attachment. Moreover, deeper penetration has antibacterial effects and also encloses living bacteria in tubules by cutting them off from nutrient sources.

The chemistry of sealers has been constantly modified as technology in each step of endodontic therapy has advanced. One such change is a calcium silicate-based sealer, which was initially launched in 2007 and has high biocompatibility and hydrophilicity. Angelus' most recently introduced sealer with the aforementioned chemistry, the BIO-C ION + sealer, offers a resin-free formula in preloaded syringes and disposable intracanal tips ready to insert into the canal. High flow aids in the sealing of difficult-to-reach places.

There isn't much information available about the properties of the BIO-C ION+ and NanoSeal-S sealers. The current study aimed to analyze and compare the dentinal tubule penetration and POBS of three distinct types of root canal sealers. In this regard, we chose and contrasted three recently created root canal sealants: BIO-C ION+, a calcium-silicate-based, NanoSeal-S, a polydimethylsiloxane-based and AH Plus a resinbased root canal sealer.

According to the results for mean dentinal tubule penetration, the increasing order among the three sealers was.

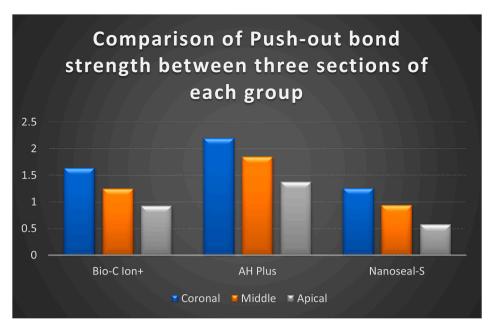
9.1. BIO-C ION+ (Group I subgroup A) > AH plus (Group II subgroup A) > NanoSeal-S (Group III subgroup A)

The increasing order for mean dentinal tubule penetration between different root sections of the same group was.

9.1.1. Coronal > middle > apical

This study's results are consistent with those of previous research by Akcay et al. (2018),²⁰ Caceres et al. (2021),²¹ and El Hachem et al. $(2019)^{22}$ who have found that bioceramic sealers had greater penetration than resin and silicone-based sealers.

The American Dental Association stipulates that a concentration of 0.1 % Rhodamine B has no impact on the flow characteristics of sealers and that any quantity larger than 0.1 % results in excessive fluorescence

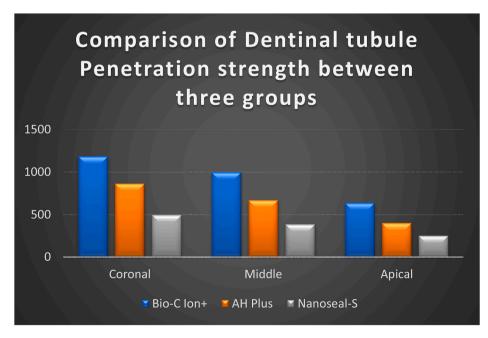


Graph 2. Comparison of push-out bond strength between three sections of each Group (Intra-group comparison).

Table 3

comparison of dentinal tubule penetration between each section of three groups (inter-group comparison).

Section Bio-C Ion+			AH Plus		Nanoseal-S		p value
	Mean	SD	Mean	SD	Mean	SD	
Coronal	1184.69	207.09	864.14	65.58	495.64	100.06	< 0.001*
Middle	997.03	165.92	669.08	47.99	384.43	71.25	< 0.001*
Apical	637.26	239.79	400.52	79.10	250.85	77.97	<0.001*



Graph 3. Comparison of dentinal tubule penetration between each section of three groups (inter-group comparison).

that makes the specimens challenging to see.²³

The sealer's penetration depth is determined by the chemical and physical characteristics of its constituent parts (including flow, surface tension, particle size, and setting time). There is no published research on the BIO-C ION + root canal sealer's dentinal tubule penetration

efficiency. The BIO-C ION + root canal sealer's better penetration in our study can be ascribed to its exceptionally minuscule particle size (>2 m) and good flow rate (22 mm–25mm), which may promote sealer flow into dentinal tubules and anatomic abnormalities. Furthermore, owing to the calcium di-silicate element, which uses the moisture in tubules to

Table 4

comparison of push-out bond strength between each section of three groups (inter-group comparison).

Section	Bio-C Ion+		AH Plus		Nanoseal-S		p value
	Mean	SD	Mean	SD	Mean	SD	
Coronal	1.64	0.18	2.20	0.23	1.26	0.22	< 0.001*
Middle Apical	1.25 0.93	0.27 0.15	1.85 1.38	0.34 0.36	0.94 0.58	0.15 0.09	$<\!\!0.001^* <\!\!<\!\!0.001^*$

commence and finish the setting phase, BIO-C ION + exhibits minimal or no shrinkage. $^{\rm 24}$

Additionally, it has been shown that the alkaline nature of bioceramic sealer by-products causes dentinal collagen fibers to denature, allowing sealers to penetrate dentinal tubules. These characteristics enable the sealer to fill the lateral canals and expand over the dentin walls of the root canals. Greater dentinal tubule penetration may result from all of these traits.

In contrast, despite having a high fluidity with a flow rate of 21.2 mm and low polymerization shrinkage, epoxy-resin-based sealers are hydrophobic, 7,25 which may impair their capacity to enter and adapt to dentinal tubule walls. Furthermore, the AH Plus sealer's typical particle size is 8–26 μ m, and the 8-h setting time explains the lower measured penetration depth.

CLSM examination revealed that the BIO-C ION + sealer penetrated thfig7e tubules smoothly and consistently, with fewer gaps (Fig. 4a, b, 4c) as compared to AH Plus (Fig. 5a, b, 5c) and NanoSeal-S (Fig. 6a, b, 6c) in all root sections. This regularity of penetration could be attributed to its hydrophilicity, single syringe system, and small particle size, which allow for deeper and more homogenous mass. In contrast, AH-Plus exhibited less evenness, with a grainy look and intratubular gaps, which could be attributed to its large particle size, shrinkage, or mixing issues. Sealer proportions and mixing processes may be critical in the tubule adaption of these materials. Arikatla et al. $(2018)^{26}$ found that AH-Plus outperformed bioceramic sealers in tubule adaptation, however, the latter were not premixed sealers. Patri et al. $(2020)^{27}$ exhibited the opposite (see Fly. 7).

Sclerosis and decreased tubule density have been observed in the apical dentin, with some places completely missing dentinal tubules²⁸, which could be the result for greatest penetration in the coronal section

in our study which is consistent with previous studies by Weis et al. (2004),²⁹ and De Deus et al. (2004).³⁰ Additionally, as we approach to the apex, smear layer removal techniques become less successful, which may prevent root canal sealers and irrigating solutions from penetrating as deeply. In the root dentin compared to the coronal dentin, dentinal tubules have more major branches.

According to the outcomes for mean push-out bond strength, the increasing order among three sealers was.

9.2. AH plus (Group II subgroup B) > BIO-C ION+ (Group I subgroup B) > NanoSeal-S (Group III subgroup B)

The increasing order for mean push-out bond strength between individual root sections of the same group was.

9.2.1. Coronal > middle > apical

In the current study, for the evaluation of POBS, three plunger sizes (apical, middle, coronal) were employed for each root third. In a study by Chen et al. (2013),³¹ the plunger tip should be 0.85 times smaller than the size of the filling substance. Furthermore, the authors have

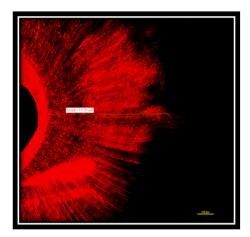
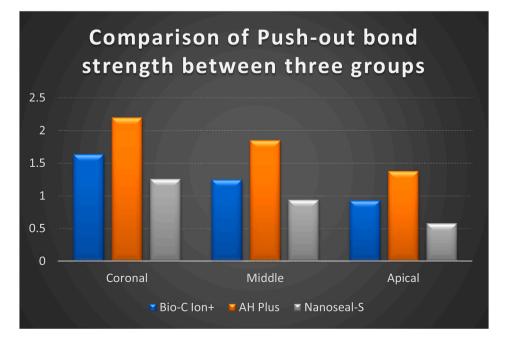


Fig. 4a. coronal.



Graph 4. Comparison of push-out bond strength between three sections of each Group (inter-group comparison).

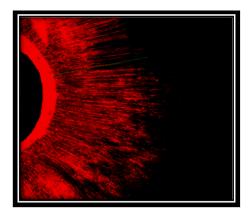


Fig. 4b. middle.

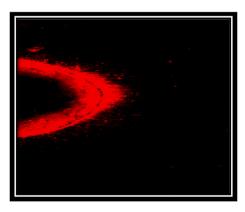


Fig. 4c. apical confocal images of group i subgroup a (bio-c ion+).

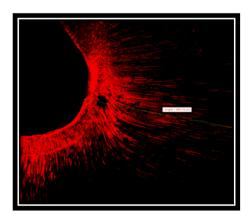


Fig. 5a. coronal.

advised that the tip of the plunger be positioned closer to the sealer's diameter. For each studied sample, pluggers of varying diameters (0.3–0.8 mm) were utilized to closely match the diameter of the root-filling substance.

The findings of this investigation are research by Donnermeyer et al. (2018)³² and Tedesco et al. (2019)³³ that AH Plus has higher POBS than calcium-silicate and silicone-based sealers. AH Plus performance is widely verified in the literature³⁴ This is due to the AH Plus's intrinsic expansion characteristic, which can generate a covalent link between the epoxy resin sealer's open epoxide ring and the exposed amino groups of radicular dentin. Furthermore, AH Plus exhibits strong cohesion between its molecules, which could translate to a high adhesion property. This can improve its mechanical interaction with radicular dentin and its

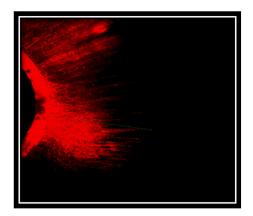


Fig. 5b. middle.

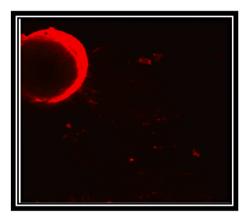


Fig. 5c. apical confocal images of group ii subgroup a (ah plus).

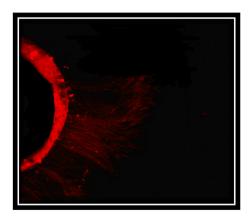


Fig. 6a. coronal.

resistance to dislodgment.

The key advantage of Calcium-silicate-based root canal sealers such as BIO-C ION+, according to the manufacturer, is that its principal components are calcium silicate and hydroxyapatite. As a result, it is both biocompatible and bioactive. The calcium silicate-based sealer and the root canal wall interact micromechanically and chemically to produce a "mineral infiltration zone," which is a weaker connection to the dentin than epoxy resins.

The obturated roots in the earlier study by Shokouhinejad et al. $(2013)^{35}$ were kept in Phosphate buffered saline for 7 days before the bond strength testing, and the authors hypothesized that with longer storage times, canals filled calcium silicate-based sealer would develop

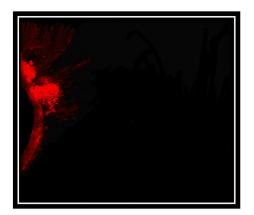


Fig. 6b. middle.

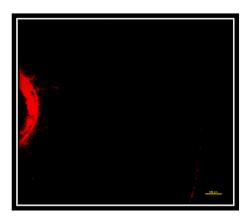


Fig. 6c. apical.

indeterminate calcium phosphate precursors along the apical root segment, this apatite deposition would be seen in the upper levels of the roots³⁶ In our investigation, samples were kept in storage for 24 h while being humidified using saline this is one of the limitation of the current study apart from being In Vitro. BIO-C ION+ is a bioactive material, so if we had used PBS the bond strength may have been enhanced. This could be another reason for lower POBS values as compared to AH Plus.

Nanoseal-S demonstrated the worst push-out bond strength in our sample. Because silicone is present and may provide significant surface tension forces that hinder the dispersion of these materials, this might be linked to the root dentin's poor wetting. According to one such study by Dem et al. (2008)³⁷ on GuttaFlow-2, which has similar components to Nanoseal-S, polydimethylsiloxane, and micro-silver, GuttaFlow-2 exhibits the lowest POBS when compared to AH Plus and GuttaFlow Bioseal.

A similar pattern was observed in the same thirds of different groups (Intergroup comparison) with the coronal third having the greatest POBS, followed by the middle and apical sections, which was consistent with earlier research by Arajo CC et al. (2015).³⁸

Regardless of the sealer used, the apical section displayed the lowest bond strength values. Several studies testing bonding techniques on root canal dentin have noted a decrease in bond strength in the apical region.³⁹ It has been demonstrated that decreased bond strength values in these regions are connected with reductions in the number and diameter of dentinal tubules in apical portions of the root canals. Additionally, the inability of irrigants to remove the smear layer in apical sections hinders the penetration of sealants into the tubules and decreases the contact between the filling material and the root dentin walls⁴⁰

Sagsen et al. (2011)⁴¹ found greater bond strength in the middle and apical sections compared to coronal specimens, which contrasts with our

study. This difference in results may be the result of the methodology used in the previous study, which involved lateral compaction, which increased compaction forces, particularly in the middle and apical sections. Cold laterally obturated root fillings, however, have been demonstrated to contain voids that could compromise the sealer's integrity or even be filled with only sealer that could eventually resorb away. Furthermore, lateral condensation with 0.02 tapered cones lacks homogeneity, is less consistent, and adapts poorly to the root walls. On the other hand, a bigger more consistent gutta-percha mass might be obtained by filling with a larger, tapered cone. Another advantage of using single-cone technology is the establishment of linear resistance in the canal, which inhibits the dislodgement of filling material. As a result, it was utilized in our study.

Apart from being an In vitro study another limitation of the present study is the use of Rhodamine B dye which is a water-soluble dye and may take up the water present in the canals to show false penetration values, strength of the current study includes the use of seamless CLSM and UTM analysis, use of single cone technique and all the calibrated steps.

10. Conclusion

Within the confines of the study, we can conclude that:

 $\rm BIO\text{-}C$ ION + showed maximum dentinal tubule penetration followed by AH Plus and NanoSeal-S. With respect to POBS, AH Plus showed maximum values followed by BIO-C ION+ and NanoSeal-S. The coronal section showed maximum values regarding both dentinal tubule penetration and POBS.

Future research is still required to assess new root canal sealers in clinical settings, despite the potential clinical importance of dentinal tubule penetration and POBS.

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A. Verma et al.

Journal of Oral Biology and Craniofacial Research 14 (2024) 143-151

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