ORIGINAL RESEARCH

Evaluation of Microleakage of Nanoparticle-incorporated Cyanoacrylate Root Canal Sealer Using the Radioisotopic Method: An *In Vitro* Study

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Abstract

Aim of the study: The study aimed to assess the microleakage of nanoparticle-based (NPB) cyanoacrylate sealer and epoxy resin-based (ERB) sealer using radioisotope method and confocal laser scanning microscopy (CLSM).

Materials and methods: A total of 100 single-rooted teeth were collected; specimens were accessed, instrumented, and irrigated, and randomly distributed into 4 groups of 25 samples each: Group I: Positive control, group II: Negative control, group III: Obturated with NPB sealer, and group IV: Obturated with ERB sealer. All samples were immersed in 99mTc pertechnetate solution, for 3 hours after which radioactivity was estimated under a Gamma camera. The radioactivity released by specimens before and after nail varnish removal was statistically analyzed. After 2 weeks, the same samples were used for CLSM analysis. The sealer tubular penetration depth was measured at the deepest level for each group using ZEN lite 2012. Data collected was statistically evaluated.

Results: The amount of radioactivity observed at first in group III and group IV was 194.76 and 599.12 units, respectively, with p-value < 0.001, indicating significant interaction, and after nail varnish removal, it was 89.68 and 468.44 units, respectively, with a p-value < 0.001; again, indicating statistical significance. Hence, the radioactivity of NPB sealer was found to be lower than ERB sealer in both cases, indicating better sealing of the former. The photomicrographs show that mean value of dye penetration in NPB sealer in first, second, and third segment from apex was 85.06, 75.73, and 66.09, respectively; while in the case of ERB sealer, those were 597.28, 461.17, and 195.68, respectively; with p-value < 0.001; signifying that NPB sealer exhibited higher resistance to microleakage than ERB sealer.

Conclusion: The NPB sealer can become a potential root canal sealer in future endodontics due to superior physiochemical properties attributed to the cyanoacrylate and incorporated nanoparticles.

Clinical significance: The study clinically signifies that we can equally use the radioisotopic method along with confocal method while conducting the microleakage studies. In addition, NPB sealer can be an emerging replacement with better properties than gold standard root canal sealers for clinical use.

Keywords: Bonding, Dentin, Microleakage, Root canal sealers.

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Introduction

The primary goal of conventional endodontic treatment is the prevention and/or elimination of apical periodontitis, a frequent complication encountered brought on by pathogenic bacteria in the root canal system. The management strategy is directed toward the eradication of micro-organisms from the root canal system and the prevention of re-infection. The most important phase of endodontic therapy is the chemomechanical preparation of the root canal using a combination of mechanical instrumentation and chemical disinfection, followed by sealing off the pulp space and the access cavity.[1]

The successful outcome of endodontic treatment is determined by the establishment and maintenance of a hermetic seal of the root canal system, which necessitates the usage of intracanal sealers that can firmly adhere to the root canal walls and the materials used for root filling.[2] An ideal root canal sealer requires that it must provide an optimal seal when set; there should be an adequate setting time to ensure the proper working time, dimensional stability, appropriate adhesion with canal walls, and biocompatibility.[3]

However, the development of voids between the sealer and dentin, may let the passage of bacteria and their byproducts and jeopardize the efficacy of the treatment. In addition, microleakage at the sealer-dentin interface might also contribute to root canal failure owing to sealers.[3–6] This can be resolved by bonding the sealer to radicular dentin and creating a monoblock. Hence,

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the type of endodontic sealer plays a critical role in the extent of bacterial leakage, which is a frequent occurrence seen in endodontically treated teeth. This raises interest concerning the standard of obturation offered by the sealing materials currently in the global dental market.[7] Thus, to overcome the shortcomings of conventional sealers, newer nanomaterials and tissue adhesives are being introduced to the field of endodontics with promising features and exceptional properties as far as surface chemistry and bonding are concerned.

In this study, nanoparticle-based (NPB) cyanoacrylate sealer and epoxy resin-based (ERB) sealer (AH-Plus; Dentsply, Germany) are evaluated for microleakage of the samples. The NPB sealer prepared using zinc oxide nanoparticles (ZnO-Np) and Chitosan nanoparticles (CS-Np) along with isoamyl cyanoacrylate which is expected to have better sealing properties and less microleakage is compared with conventional gold standard sealer (ERBS). The NPB sealer, comprising of chitosan and zinc oxide as nanoparticles were tested for microleakage and assessed and evaluated with ERB sealer. Numerous techniques, including bacterial penetration, fluid transfer, radioisotope clarifying penetration, electrochemical approaches, and gas chromatography, are available for assessing the effectiveness of root canal sealers. Clarification penetration of radioisotopes is a novel methodology introduced recently. The current study is designed to assess and analyze the microleakage of the newly introduced NPB sealer and the gold standard ERB sealer (AH Plus, Dentsply, Germany) using the radioisotopic dye penetration method and confocal laser scanning microscopic analysis. The null hypothesis tested was that there are no variations in the microleakage between the sealers groups.

Materials and Methods

Sample Selection

Fifty-four extracted human single-rooted permanent teeth were collected for the study. The samples were selected after radiographic evaluation of single roots. The inclusion criteria consisted of straight roots with fully developed root apices, free of caries, cracks, resorption, or any other previous treatment. Samples were handled according to OSHA regulation.[8]

Materials Used

The two sealers used in the current study were NPB cyanoacrylate sealer and ERB sealer (AH-Plus; Dentsply, Germany). The NPB is composed of cyanoacrylate and nanoparticles, chitosan, and zinc oxide.

For preparing NPB sealer, CS-NPs, zinc oxide nanoparticles, zinc oxide powder, and barium sulfate were included in the powder component, which was prepared according to the percentage ratio. The liquid contained glycerin and isoamyl 2 cyanoacrylate. Both nanoparticles (chitosan (SRL 65468 HSN Code: 29211990) and zinc oxide nanoparticles (SRL 60345. UN No.:3077, IMCO Class No.:9) were incorporated into the powder together, along with the other components. The powder-to-liquid ratio of 40 mg of powder and 2 mL of liquid was considered ideal for preparing the sealer.[9] The ideal sealer consistency was achieved by mixing the powder and liquid.[10] The AH Plus sealer was mixed according to the manufacturer's instructions in a completely sterile environment.

Specimen Preparation

Teeth were decoronated to standardize the root length to 12 mm and canals were accessed. Working length was determined by placing a #10K (Mani Inc., Japan) file into each root canal until it was just visible at the apical foramen and then subtracting 1 mm from this point.[11] Instrumentation was performed at a working length equivalent to 11 mm following a crown-down technique with rotary files (Protaper Universal) up to F3. A 10 mL of freshly produced solution of 5.25% sodium hypochlorite (Vensons, Bengaluru, India) (NaOCl) and 17% EDTA (Septodont, Cedex, France) solution was used to irrigate canals in a cycle that alternated between files; final rinse was performed with normal saline followed by drying of the canals using paper points.[11] The prepared samples were randomly distributed into four groups. Scoring was done by two observers randomly and average reading was recorded as final values.

Group I: Positive Control ($n = 2$) (Obturated with F3 gutta-percha as core material, without sealer)

Group II: Negative Control ($n = 2$) (Obturated with an F3 gutta-percha cone without sealer). The roots were then totally coated with 2 layers of nail polish, including the apical foramina, to ensure that there was no leak of fluid movement anywhere within the device.

Group III: ($n = 25$) (Obturated with NPB sealer with F3 gutta-percha as core material)

Group IV: ($n = 25$) (Obturated with ERB sealer with F3 gutta-percha as core material)

Restorative glass ionomer cement (GC India) was used to seal the coronal opening. The samples were kept in an incubator at 37°C for 24 hours; then subjected to thermocycling with 500 cycles between 5 and 55°C with a dwell time of 30 seconds.[13] After the drying of sample surfaces, two coats of colored nail varnish were applied to the external surface of the root two millimeters from the apex. The root surfaces of the negative control teeth were entirely coated with two layers of nail varnish to prevent possible leakage.

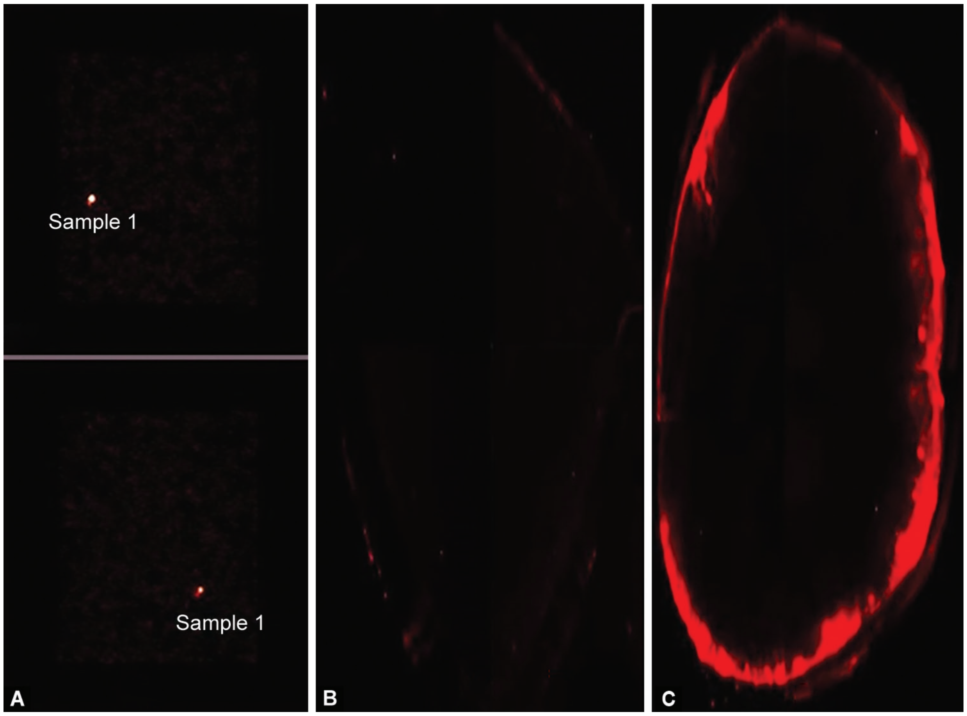
Specimen Preparation for Microleakage Evaluation

Radioisotope Dye Penetration Method

Specimens of all groups were immersed in 99mTc pertechnetate (Na^{+} ^{99m}Tc O4-) solution, for 3 hours. Technetium is the lightest radioactive element, produced by the radioactive decay of molybdenum.[12] The specimens were made to undergo sequential dilutions by passing through serial water baths. Then the radioactivity was estimated under Gamma camera (Millennium MPR, Wipro GE Health Care Pvt Ltd) and noted as before reading, as it was recorded before removal of the nail varnish, which was having the absorbed 99mTc dye. The nail varnish was removed from the samples and the radioactivity was evaluated under a Gamma camera as after reading (reading taken after removal of the nail varnish).[12] After that, the specimens were stored in the nuclear medicine storeroom for 2 weeks to avoid any radiation leakage. After 2 weeks, the same samples were used for confocal laser scanning electron microscopic analysis. The radioactivity released by the specimens before and after were statistically analyzed (Fig. 1A).

Specimen Preparation for Confocal Laser Scanning Microscopic Analysis

The samples received from nuclear medicine storeroom were again coated with two layers of same nail varnish used for radioisotopic study and allowed to dry. After that the samples were immersed in Rhodamine B dye (0.1 mg/mL) for 24 hours. After 24 hours, the samples were rinsed with the water.[13]



Figs 1A to C: (A) Readings obtained in the Gamma camera; (B) CLSM representative images of the sealer microleakage for NPB; (C) ERB sealer

Each test sample was moulded and covered in self-curing resin before being mounted and sectioned perpendicular to its long axis on a Metsaw (RB205 Metsaw-LSTM; R&B, Daejeon, Korea) equipped with a low-speed diamond wheel and a continuous water-cooling system. The horizontal cutting point was established at 3, 5, and 7 mm from the apex, and a slice measuring 0.1 mm thick was obtained.[14]

The Vectashield Antifade Mounting Medium (Vector Laboratories, Inc., Burlingame, CA, USA) was used to mount all collected specimens onto a slide glass for CLSM observation (Zeiss LSM 880, Germany). To visualize and obtain correct images, pictures were observed and recorded using a 20× magnifying lens. The ZEN imaging software's tile scan function was used to capture several fragmentary photos, which were then combined into a single full-specimen image utilizing the stitching feature (Carl Zeiss Microscopy GmbH).[14] Rhodamine B's excitation and emission wavelengths were chosen at 558 and 575 nm, respectively. Using ZEN lite 2012 (Carl Zeiss Microscopy GmbH, Jena, Germany), the deepest dentinal tubule penetration length of each sealer was determined (Figs 1B and 1C). Data collected were statistically evaluated.

Statistical Analysis

The *t*-test was applied to analyze the results. The descriptive analysis for quantitative variables was expressed as arithmetic mean standard deviation after the values of the average counts of each tooth was obtained. Using the statistical program SPSS9.0 for Windows (SPSS Inc, Chicago, IL, USA), statistical significance was established in advance as $p < 0.05$.

Results

Microleakage Evaluation by Radioisotope Method

The samples in group I as the positive control, showed maximum radioactivity indicative of the importance of root canal sealer for obturation while the samples in group II (negative control) did not show any radioactivity. This signifies the effectiveness of nail varnish in inhibiting the penetration of the dye into the restorative surface of the sealer. Table 1 shows the amount of radioactivity released by the specimens before and after nail varnish removal for group III and group IV. The amount of radioactivity observed at first in group III and group IV samples is 194.76 ± 15.29 and 599.12 ± 17.07, respectively, indicating significant interaction. The radioactivity of the NPB sealer is lesser than the ERB sealer; indicating the better sealing of NPB sealer. The amount of radioactivity observed after the removal of nail varnish in group III and group IV is 89.68 ± 7.43 and 468.44 ± 30.46, respectively, indicating significant interaction. The radioactivity of NPB sealer is lesser than the ERB sealer even after the removal of nail varnish, again indicating the better sealing of NPB sealer. When the radioactivity was compared between the before and after samples, there was a difference of 105.08 ± 18.62 units of activity in group III. However, it was 130.68 ± 37.68 units in the case of group IV; indicating that NPB sealer has better sealing ability than ERB sealer.

Confocal Laser Scanning Microscopic Analysis

The samples in group I as the positive control, showed maximum dye penetration indicative of the importance of root canal sealer for obturation while the samples in group II (negative control) did not show any dye penetration. This signifies the effectiveness of nail varnish in inhibiting the penetration of the dye into the restorative surface of the sealer.

The results show that the mean value of dye penetration in the coronal third segment from the apex in NPB sealer was 85.06 ± 3.10; whereas in ERB sealer, the mean value was 597.28 ± 35.25 (Table 2). Similarly, the mean value for the middle third segment from the apex in NPB sealer was 75.73 ± 2.52; whereas in ERB sealer, the mean value was 461.17 ± 32.92. The apical third segment from the apex in NPB sealer was 66.09 ± 3.88; whereas

Table 1: Mean values and standard deviations of the radioactivity before and after nail varnish removal for group III (NPB) and group IV (ERB)

					95% Confidence interval of the difference				
Group	*N*	*Mean*	*Std. deviation*	*Mean difference*	*Lower*	*Upper*	*T*	*Df*	*p-value*
Before									
III	25	194.76	15.29	−404.36	−413.57	−395.15	−88.23	48	<0.001*
IV	25	599.12	17.07						
After									
III	25	89.68	7.43	−378.76	−391.37	−366.15	−60.40	48	<0.001*
IV	25	468.44	30.46						
Change									
III	25	105.08	18.62	−25.60	−42.50	−8.70	−3.05	48	0.004*
IV	25	130.68	37.68						

Independent sample *t*-test. $p > 0.05$ non-significant, *$p < 0.05$ statistically significant

Table 2: Mean values and standard deviations of dye penetration at the apical, middle, and coronal levels for group III (NPB) and group IV (ERB)

					95% Confidence interval of the difference				
Group	*N*	*Mean*	*Std. deviation*	*Mean difference*	*Lower*	*Upper*	*T*	*Df*	*p-value*
A (Coronal)									
III	25	85.06	3.10	−512.21	−526.44	−497.98	−72.38	48	<0.001*
IV	25	597.28	35.25						
B (Middle)									
III	25	75.73	2.52	−385.44	−398.72	−372.16	−58.37	48	<0.001*
IV	25	461.17	32.92						
C (Apical)									
III	25	66.09	3.88	−129.59	−135.74	−123.44	−42.38	48	<0.001*
IV	25	195.68	14.79						

Independent sample *t*-test. $p > 0.05$ non-significant, *$p < 0.05$ statistically significant

in ERB sealer, the mean value was 195.68 ± 14.79. These results show that the apical microleakage is lesser than the middle and coronal portion of the root, in both groups. Also, the NPB sealer exhibited less microleakage than the ERB sealer in all the segments of the tooth samples. Furthermore, within the obtained values we have observed that NPB sealer had better sealing ability, with less microleakage values in both the methodologies when compared.

Discussion

The microbial invasion along the root canals is prevented by a good three-dimensional obturation of the root canal and applying an endodontic sealer to fill the voids at the gutta-percha/dentin interface.[15] The selection and manipulation of the sealer are very important criteria for apical microleakage in endodontically treated teeth. Even though several methodologies are present in evaluation of microleakage, most widely used was confocal laser microscopic technique. But the speed of image acquisition is a major drawback of point-scanning confocal microscopy for live cell imaging applications, as it may be insufficient to gather data on rapidly changing biological processes.[16] In nuclear medicine, the radioisotopic dye penetration technique, is a very popular *in vitro* method for evaluation of microleakage as it is relatively easy and rapid by allowing the tracer agent to penetrate the filled canal.[17] In this study, an attempt is done to include the newer methodology to compare the microleakage of novel endodontic sealer with the gold standard root canal sealer under standard laboratory conditions.

In the present study, the results revealed no leakage in the negative control group, confirming that two coats of nail varnish effectively prevented dye penetration, while the positive control group showed the greatest dye penetration, indicating the efficacy of dye penetration in leakage studies.

In the current study, the experimental NPB sealer was tested and compared with the ERB sealer. The ERB sealer is one of the most widely used ERB sealers to date and is regarded as the gold standard for evaluating new sealers due to its superior sealing capabilities, high strength, dimensional stability, and low solubility.[12] However, when compared with NPB sealer, it exhibited higher microleakage at all the segments from the apex. This might be attributed to the larger particle size in the resin-based sealer.[14]

The NPB sealer used in the present study is a cyanoacrylate-based cement incorporated with nanoparticles, chitosan, and zinc oxide. The cyanoacrylate can become a potential material for sealer since it can be used in the form of a dentin adhesive for bonding to intraradicular dentin. With the tooth as a substrate, cyanoacrylate exhibits a chemical adhesive mechanism. When compared with conventional dental cement, it has a unique bonding process. With a strong tendency for bonding to basic substances, it hardens by the anionic polymerization of calcium ions into dentin by cyano and carbonyl groups found in cyanoacrylate.[18] The two nanoparticles, chitosan and zinc oxide were incorporated to improve the physiochemical and antimicrobial properties of a sealer. A natural polycationic linear polysaccharide, chitosan is chemically made up of randomly distributed ß-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. It is created by deacetylating chitin compounds, which make up the majority of crustacean animals' shells, including crabs and shrimp.[19] Nanoparticles of Chitosan (CS-NPs) have been developed recently, being extensively researched in the area of endodontics, with the ability to enhance physiochemical and antimicrobial characteristics. Based on other studies, the chitosan nanoparticles when used with a root canal sealer enhance sealer adaptation, thereby improving the apical sealing.[20] Also, it has been observed that adding CS-NPs to the zinc oxide-eugenol sealer prevents the formation of biofilm at the sealer-dentin interface.[21]

Additionally, since chitosan is hydrophilic, it can closely adhere to intraradicular dentine resulting in an effective absorption into the canal wall. Since it has many hydroxyl and amino groups, ionic interactions take place readily with dentinal calcium ions. The amino group in chitosan can be protonated, which allows it to penetrate deeper into the dentinal tubules and attract more molecules out for adsorption into the radicular dentine.[22]

Another nanoparticle that was incorporated in NPB is zinc oxide (ZnO-Np). Based on the studies, this modification inhibits biofilm formation within the sealer dentin interface, reduces cytotoxicity, and improved the sealing ability and antibacterial properties of NPB sealer. This will reduce or stop the growth of microorganisms at the sealer and tooth interface.[23] Hence, compared with ERB, NPB demonstrated lesser microleakage. The results can be supported by another study where it was observed that in the apical third region of the root, ERB sealer with the inclusion of CS-NPs showed fewer gaps than the same without. The interaction between the ERB sealer and the dentine wall was improved by the incorporation of CS-NPs relative to other groups.[24] The usage of sealers with optimal qualities like adaptability, adhesion, and tubular penetration enables superior root canal sealing due to the increased sealer contact with the radicular dentine and encapsulation of remaining microbes in the tubules. Additionally, a study by Ramar et al.; demonstrated that CS-NPs are biocompatible with fibroblasts and that they can be utilized in conjunction with root canal sealants in primary teeth.[25]

In the case of ERB, which is considered a gold standard, it is crucial to stress that AH Plus sealer penetrates more deeply into surface micro-irregularities and inside lateral root canals due to its resin composition, flow, and lengthy curing time.[26] These characteristics cause the sealer and dentin structure to become more intertwined, which, along with the cohesion among the cement molecules, increases the adhesion and resistance to dislodgment from dentin.[27] However, even after such bonding mechanisms, ERB exhibited greater microleakage than NPB sealer, indicating superior adaptation of NPB sealer due to cyanoacrylate and the incorporated nanoparticles.

In radioisotopic dye penetration method, the initial values have shown lesser radioactivity of NPB sealer ($p < 0.0001$) indicating better sealing of the same. The values were initially taken before the removal of nail varnish which indicates the combined radioactivity measured on the nail varnish and the restorative area of the samples. The values were finally taken after the removal of nail varnish which indicates the radioactivity on the restorative areas of the samples. After the removal of nail varnish, the radioactivity of the NPB sealer is lower than the ERB sealer ($p < 0.0001$), again indicating the better sealing of the NPB sealer. This concludes that the microleakage in NPB sealer is lesser than the microleakage observed in ERB sealer. This can be attributed to the composition of NPB sealer which includes cyanoacrylate and nanoparticles. Cyanoacrylate's adhesive property aids in the sealer's improved adhesion to the dentinal surfaces. A study found that when incorporated in root canal sealers, such as zinc oxide-eugenol, showed significant decrease in microleakage.[28] Another study done by Bernardes et al., suggested that the filler particle size affects the flow of sealer; the lesser the size, and the higher the flow. With the increase in the sealer flow, there is an increased penetration of sealer to uneven surfaces and root canals, as can be seen in the case of NPB.[24]

In confocal laser microscopic method, NPB sealer exhibited the lesser microleakage when compared with the ERB sealer. This indicates the similarity of the results obtained from radioisotopic method. Upon considering the apical, middle, and coronal sections of confocal method; the apical section exhibited the least microleakage than the coronal section. This finding was along expected lines and could be ascribed to one or more of the following reasons: greater diameter and number of the dentinal tubules coronally, lesser tubule density apically, propensity of sclerotic dentin to form toward the apical region, the reduced effectiveness of irrigants at the apex of the root canal and greater lateral compacting forces coronally during obturation. These findings are in accordance with the study done by Mokashi et al.[14]

The method used for the detection of microleakage in the current study was the radioisotope penetration method. This had been recently used for the evaluation of microleakage of restorative materials. This methodology was compared and assessed with the confocal laser scanning electron microscopic analysis of the same samples to avoid bias. Several radioactive isotopes, including the markers ^{45}Ca and ^{131}I have been utilized in microleakage research. Since Technetium-99m (99mTc) is the radionuclide that is utilized the most in the area of nuclear medicine, it was chosen for this study. This radioactive technology allows for the quantitative evaluation of microleakage from the same samples at intervals over long periods without causing any damage to them.[29,30] This radionuclide was selected because it has a half-life of around 6 hours and is the cold kit that is most frequently used in nuclear medicine for single photon emission. To allow time for the above-mentioned procedures and the gamma camera radiation measurement, 3 hours here chosen as the duration for which the teeth were immersed in the sodium pertechnetate solution. To avoid any contamination after the immersion time, the samples were meticulously prepared for gamma camera quantification following immersion. Each sample's radioactivity was accurately measured using a gamma camera. The results obtained in this study, comparing both methodologies are in the same frequencies. This indicates that the radioisotopic dye penetration method can be effectively used for the analysis of microleakage of root canal sealers.

The limitation of this study is that it is an *in vitro* study and performed under controlled laboratory conditions. Moreover, the present study is done on single-rooted teeth and further similar studies are required for multirooted teeth.

Within the limitations of this *in vitro* study, the NPB sealer tested demonstrated greater resistance to microleakage as compared with the ERB sealer. Considering the superior physiochemical and antibacterial properties of the NPB sealer, it seems close to fulfilling the ideal requisites of a root canal sealer. The radioisotopic dye penetration method supplemented with confocal laser scanning microscopy validates the results of the study. However, long-term clinical studies may have to focus on the precise determination of the degree of sealing by the obturation materials and the physiochemical properties utilizing more specific and innovative methods of evaluation.

Clinical Significance

Microleakage at the sealer-dentin interface is a major contributing factor of root canal failure. Hence, the type of endodontic sealer plays a crucial role in the extent of bacterial leakage. The cyanoacrylate-based NPB sealer used in the present study, incorporated with nanoparticles, can become a potential sealer as it can be used in the form of a dentin adhesive for bonding to intraradicular dentin. Furthermore, the two nanoparticles, chitosan and zinc oxide improve the physiochemical and antimicrobial properties of a sealer. The present study was to compare the two methodologies with different root canal sealers. The radioisotopic method had similar result on same samples tested for confocal method. The study clinically signifies that we can equally use the radioisotopic method along with confocal method while conducting the micro leakage studies. In addition, NPB sealer can be an emerging replacement with better properties than gold standard root canal sealers for clinical use.

Conclusion

The NPB sealer, exhibiting lesser microleakage when tested with both methodologies, can become a potential root canal sealer in future endodontics due to superior physiochemical properties attributed to the cyanoacrylate and incorporated nanoparticles.

Ethical Approval

The ethical clearance for the study was acquired from the Central Ethics Committee of NITTE Deemed to be University (Ref. ABSM/EC/125/2021). The study was performed between August 2022 to November 2022.

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