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Original Article

Dentistry Section

Effect of Calcium Hydroxide Based Intracanal Medicaments on the Apical Sealing Ability of Resin Based Sealer and Guttapercha Obturated Root Canals

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ABSTRACT

Introduction: Calcium Hydroxide (CH) is one of the most commonly used intracanal medicaments which can be used with various vehicles.

Aim: The aim of this in vitro study was to evaluate the effect of three CH based intracanal medicaments on the apical sealing ability of AH Plus – guttapercha obturation.

Materials and Methods: Crowns of 100 extracted single rooted human teeth were sectioned at the Cemento-Enamel Junction (CEJ) to a standardized length. The root canals were instrumented upto ISO size 40 using step back technique and the specimens were randomly divided into two control and four experimental groups. The control groups were not medicated. Specimens in positive control group (Group I) were obturated with guttapercha without placing sealer and in negative control group (Group II) were obturated with guttapercha and AH Plus sealer. Among the experimental groups, specimens of Group III were not medicated while groups IV, V and VI were medicated with CH-saline, CH-2% Chlorhexidine (CHX) and Vitapex respectively for a period of 14 days. The medicaments were removed from the specimens and the teeth were obturated with AH Plus sealer and guttapercha using lateral compaction technique. The specimens were immersed in India ink dye, demineralized and diphanized. The extent of dye penetration was assessed using a 10X stereomicroscope. Data obtained was statistically analyzed by one-way ANOVA ($p<0.05$) followed by Post-hoc Tukey test.

Results: Amongst the three CH medicaments, CH-2% CHX when used as an intracanal medicament showed a significantly higher microleakage as compared to the other groups with $p<0.001$. The microleakage values between the remaining groups were not statistically significant.

Conclusion: Under the conditions of this study it was concluded that all groups with or without intracanal medicament showed apical leakage. The vehicle used to carry CH may significantly influence the apical sealing ability of guttapercha – AH Plus obturated canals.

Keywords: AH Plus, Chlorhexidine, Saline, Vitapex

INTRODUCTION

Calcium Hydroxide was introduced in endodontics as a direct pulp-capping agent and is highly recommended and widely accepted as an inter-appointment intracanal endodontic dressing. It demonstrates a pronounced antibacterial activity against most of the bacterial species identified in endodontic infections. It can be mixed with a variety of vehicles such as distilled water, saline solution, propylene glycol and glycerine. Most of the substances used as vehicles do not have significant antimicrobial activity. However, due to the relative inefficiency of CH in the elimination of both facultative anaerobes and yeasts, it has been combined with other medicaments such as 2% CHX gel, Iodoform, Camphorated Paramonochlorophenol (CPMC), to obtain a wide spectrum antimicrobial action [1].

The merits of CH have also been disputed, not only concerning its efficacy as an antimicrobial agent, but also because of possible apical leakage of the obturated canal system after its use [2]. Incomplete removal of CH medicaments from root canal surface, prevents the sealer from penetrating into the dentinal tubules, interferes with the normal setting reaction resulting in potential reduction of sealer adaptation, thus, affecting the seal of obturating material leading to microleakage and subsequent treatment failure [3]. Hence, while placing an intracanal medicament it is important to consider its effect on leakage of the root canal system [4].

The purpose of this study was to evaluate the effect of three CH based intracanal medicaments on the apical sealing ability of AH Plus–guttapercha obturation. The medicaments used in the study were freshly prepared paste of CH mixed with normal saline, freshly prepared paste of CH mixed with 2% CHX solution and a commercially available paste of CH and iodoform in silicone oil–Vitapex. Hypothesis tested was that CH based intracanal medicaments would adversely affect the apical sealing ability of AH Plus guttapercha obturated root canals.

MATERIALS AND METHODS

This in vitro study was carried out in the Department of Conservative Dentistry and Endodontics, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India. Hundred permanent single rooted, non-carious human teeth with intact apices and curvature less than 10 degrees extracted for periodontal or orthodontic reasons were selected for the study. Teeth with immature root apices, cracks, root caries, curvatures, fracture and resorption defects were excluded. Samples were disinfected in 5% sodium hypochlorite (PDP, India) solution for one hour and stored in 0.9% normal saline (Althea Pharma Pvt. Ltd. India) in air tight containers until use.

Specimen Preparation: To ensure that all specimens were the same length they were resected 15 mm from the apex using a diamond disc with water coolant. The length was standardized

[Table/Fig-1]: Clearing of teeth.

at 15 mm with the help of a Vernier's Calliper (H.M & Company, Mumbai). Working length was determined 1 mm short of the apex. To simulate clinical situation, the apex was sealed with sticky wax.

Root Canal Preparation: Mechanical instrumentation of the root canals was done to master apical file #40 with 0.02 tapered stainless steel K-file. Canals were enlarged using step back technique till #60. Circumferential filing was done using a #40 H-file to obtain uniform apicocervical taper of the canal walls and eliminate the irregularities created during step back preparation. Smear layer was removed using 1 ml of 17% EDTA (Dentwash PDP, India) followed by 5 ml of 5% sodium hypochlorite, both agitated using passive ultrasonic irrigation (P5 Booster Suprasson, Satelec/Acteon, India). Finally the canals were irrigated with 5 ml of normal saline. The sticky wax was then removed from the apex and canals were dried with absorbent points (Mani, Japan). The prepared roots were randomly divided into six groups, two control groups (n=10) and four experimental groups (n=20).

Control Groups

Group I (Positive Control): Following instrumentation, the roots were obturated with laterally compacted guttapercha without sealer and the access cavities were sealed with Cavit G (3M ESPE, Germany).

Group II (Negative Control): The prepared roots were obturated with AH Plus (Dentsply, Germany)-guttapercha using lateral compaction technique and the access cavities were sealed with Cavit G.

Following obturation, radiographs were taken to ensure that the obturation material had been placed completely throughout the canal length and width. The teeth were stored in an incubator (LabHosp, Mumbai) at 37°C and 100% relative humidity for 72 hours. For the dye penetration test the roots in Group II were completely coated with three layers of nail varnish (Revlon, India); whereas, the roots in Group I were not coated with nail varnish.

Experimental Groups

Group III: Root canals were obturated with AH Plus- guttapercha using lateral compaction technique and access cavities were sealed with Cavit G without any prior placement of CH based intracanal medicament.

Group IV (Calcium Hydroxide-Normal Saline): Powder of chemically pure CH (Depashree Products, India) was mixed with normal saline on a glass slab at a powder liquid ratio 1:1.5. The paste was then introduced into the canal with a #25 lentulospiral (Mani, Japan) followed by dry CH powder, carried by an amalgam carrier and packed with a finger plugger (Mani, Japan) until the paste extruded beyond the apical foramen. Once the apical third was filled, remainder of the canal was filled upto the orifice using hand pluggers (Mani, Japan).

Group V (Calcium Hydroxide-2% Chlorhexidine Solution): Powder of chemically pure CH was mixed with 2% CHX solution (Ultradent, India) on a glass slab to a creamy consistency. CH powder (2 gm) was used per milliliter of 2% CHX solution. The same procedure was carried out till the entire canal was filled.

Group VI (Vitapex): Vitapex (J Morita, Japan) was injected into the root canal until the material was seen extruding through the apex.

The dense packing of medicaments placed was confirmed with a radiograph and the canals were sealed with 1 mm cotton pellet and a 2 mm layer of temporary filling material, Cavit G. After that the roots were stored at 37°C and 100% relative humidity for 14 days. The CH medicament in groups IV, V and VI was removed using hand K-file #40 in conjunction with copious irrigation with 5.25% sodium hypochlorite and 17% EDTA agitated using passive ultrasonic irrigation. The canals were then irrigated with 5 ml of normal saline. The root canals were dried and obturated with AH Plus sealer and guttapercha cones using lateral compaction technique and sealed with 2 mm of Cavit-G. Teeth were stored for 72 hours at 37°C and 100% relative humidity in an incubator to allow the sealer to set. Roots in Group III, IV, V and VI were coated with three layers of nail varnish except at the apical 2 mm.

Dye Penetration Test: The teeth were immersed in India ink [5] (Rotring, Germany) and placed in the incubator at 37°C for one week. After removal from the ink, the roots were thoroughly washed in running tap water to remove the dye from all external surfaces of the teeth. The nail varnish was gently scraped from the root surface with the help of a scalpel. The roots were then demineralized in 5% nitric acid (Amrut Industrial Products, India) for three days. The teeth were washed in running tap water for four hours. They were dehydrated in ascending orders of alcohol: 80% ethyl alcohol overnight, 90% ethyl alcohol for one hour and in absolute alcohol for three hours. Following dehydration, the teeth were transferred to methyl salicylate (Amrut Industrial Products, India) for two hours

Group	N	Mean	SD	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Group I: Positive control	10	3.66	1.07	2.90	4.42	2.00	5.70
Group II: Negative control group	10	0.05	0.11	0.00	0.13	0.00	0.30
Group III: Without CH based intracanal medicament	17*	0.63	0.30	0.48	0.78	0.30	1.40
Group IV: CH in normal saline	20	0.94	0.69	0.62	1.26	0.10	2.50
Group V: CH in 2% CHX gel	20	2.47	1.40	1.81	3.12	0.40	6.10
Group VI: Vitapex	20	0.64	0.86	0.23	1.03	0.00	3.60
Total	97	1.32	1.41	1.04	1.61	0.00	6.10

[Table/Fig-2]: Descriptive statistics for the penetration of dye in different groups.
*3 samples in Group III were not assessed due to procedural errors (Insufficient clearing), CH-Calcium Hydroxide, CHX-Chlorhexidine

to allow diphanization or until the teeth were cleared [Table/Fig-1]. The teeth were dried and analyzed under 10X stereomicroscope (Labomed CSM2) to assess the extent of dye penetration in each group expressed in millimeters [6] [Table/Fig-2].

STATISTICAL ANALYSIS

Since the data distribution was found to be normal using the Kolmogorov Smirnov test [Table/Fig-3], statistical analysis was carried out by one–way ANOVA [Table/Fig-4] followed by Post-hoc Tukey test [Table/Fig-5]. The level of significance was set at $p< 0.05$.

RESULTS

The mean microleakage values and standard deviation for tested medicaments are given in [Table/Fig-2]. The specimens of Group I, positive control group showed complete leakage throughout the length of the root canal (3.66±1.07 mm) [Table/Fig-6]; whereas, Group II, the negative control teeth revealed minimal leakage (0.05±0.11 mm) [Table/Fig-7]. Among the medicated groups, Group VI medicated with Vitapex showed the least leakage [Table/Fig-8] (0.64±0.86 mm) and the Group V medicated with CH-2% CHX showed the highest leakage (2.47±1.40 mm) [Table/Fig-9]. In Group III [Table/Fig-10], the mean penetration of dye was (0.63±0.30 mm) and in Group IV, the mean penetration was (0.94±0.6 mm) [Table/Fig-11]. Three samples in Group III were not assessed due to procedural errors (insufficient clearing).

Group	Kolmogorov-Smirnov		
	Statistic	df	p-value
Group I	0.196	10	0.201
Group II	0.478	10	0.200
Group III	0.172	17	0.197
Group IV	0.230	20	0.207
Group V	0.180	20	0.287
Group VI	0.255	20	0.258

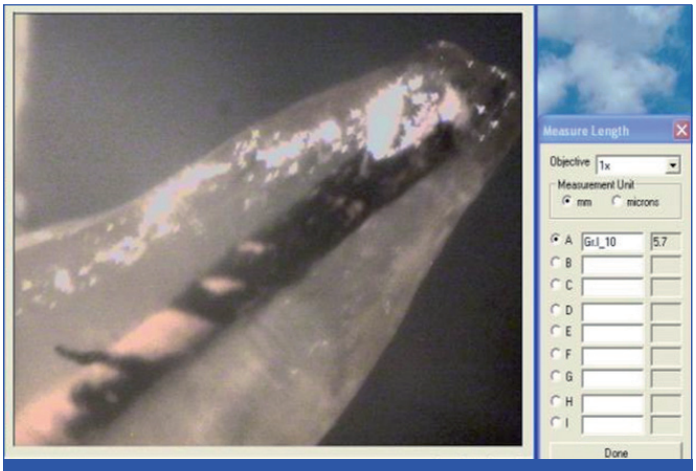
[Table/Fig-3]: Test for normality.

CP	Sum of Squares	df	Mean Square	F	p-value
Intergroup	117.621	5	23.524	29.617	<0.001
Intragroup	72.280	91	0.794		
Total	189.901	96			

[Table/Fig-4]: One-way ANOVA.

Group	N	Subset for alpha = 0.05		
		1	2	3
Group II	10	0.0500		
Group III	17	0.6294		
Group VI	20	0.6300		
Group IV	20	0.9400		
Group V	20		2.4650	
Group I	10			3.6600
Sig.		0.084	1.000	1.000

[Table/Fig-5]: Tukey Post-hoc test (Tukey HSD[a,b]).



[Table/Fig-6]: Stereomicroscopic photograph of Group I positive control following dye penetration (Group I sample no.10: 5.7 mm).

DISCUSSION

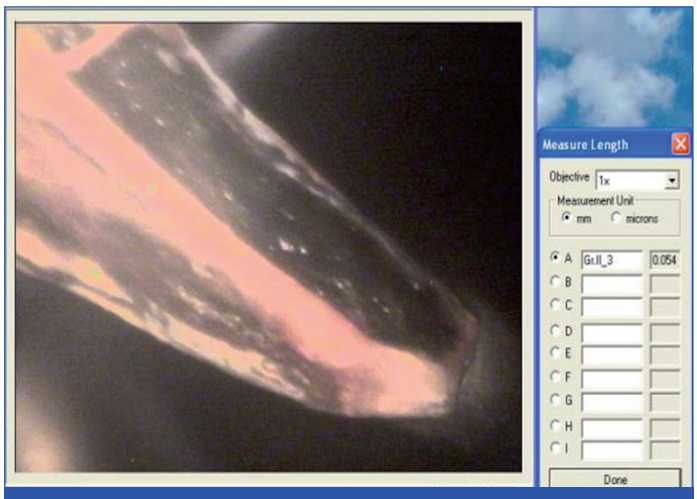
Successful root canal therapy requires thorough removal of intracanal medicaments from the dentinal walls to ensure a three dimensional seal of the root canal system at the apical dentino-cemental junction. It has been well documented that residual medicament might compromise the adaptation of filling materials and hence, the seal of the obturated root canal [7]. This inadequate sealing could result in ingress of microorganisms or tissue fluids into the canal space and induction of periapical inflammatory reaction. Hence, it is of paramount importance that the apical sealing ability of commercially available endodontic sealers be adequately evaluated to ensure long term endodontic success.

At present, epoxy resin based sealers have gained popularity due to very good physical and biological properties. Hence, in the present study AH Plus, an epoxy resin based sealer was used for obturation [8]. The teeth were stored in an incubator at 37°C and 100% relative humidity for 72 hours to allow the sealer to set.

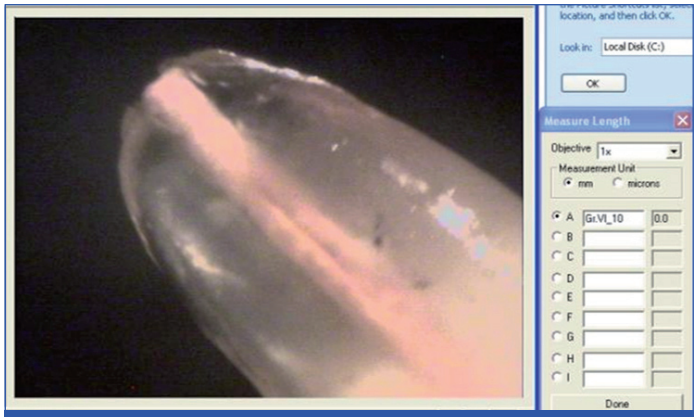
It has been reported that 17% EDTA when used for one minute followed by 5% sodium hypochlorite [9] has proven to be one of the most effective combinations in removing CH based intracanal medicaments. On the other hand, the application of EDTA for more than one minute [10] and in volume more than 1 ml has been reported to be associated with dentinal erosion. Among these protocols irrigation with 17% EDTA in conjunction with passive ultrasonic irrigation has shown promising results on debris and smear layer removal [11].

The optimal time period needed for CH to disinfect the root canal system is still not known. Several factors such as presence or absence of root canal exudate, the type of microorganisms, location of the microorganisms in the root canal system, presence or absence of smear layer and especially degree of susceptibility to the medication may markedly influence the efficacy of CH as found by Gomes BP et al., [12]. Studies have reported that CH could take upto 10 days to disinfect dentinal tubules infected by facultative bacteria as reported by few authors [13,14]. Hence, in the current study CH was placed for a period of 14 days.

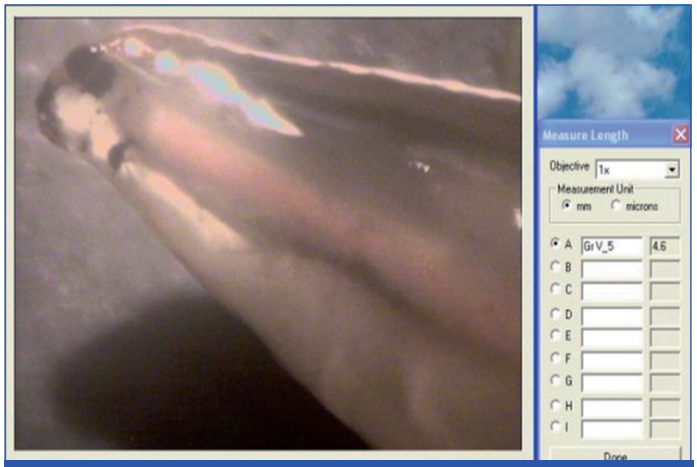
It was observed in our study that all experimental groups in which CH



[Table/Fig-7]: Stereomicroscopic photograph of Group II negative control following dye penetration (Group II sample no 3: 0.05 mm).

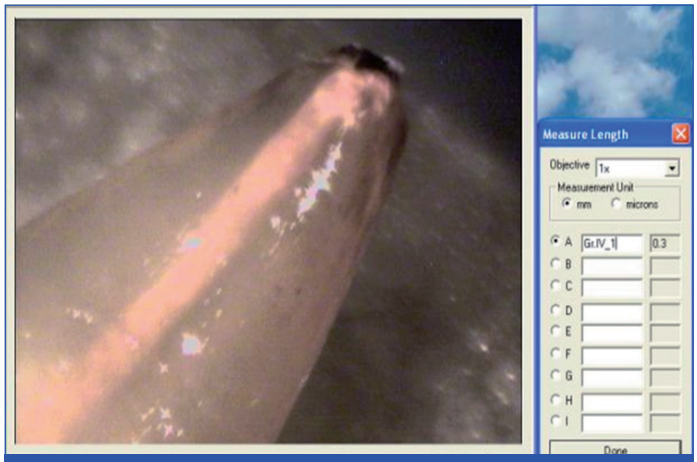


[Table/Fig-8]: Stereomicroscopic photograph of Group VI Vitapex following dye penetration (Group VI, sample No 10: 0.00 mm).



[Table/Fig-9]: Stereomicroscopic photograph of Group V CH-2% CHX following dye penetration (Group V, sample no 5: 4.6 mm).

[Table/Fig-10]: Stereomicroscopic photograph of Group III (without medicament) following dye penetration (Group III, sample no 17: 0.7 mm).



[Table/Fig-11]: Stereomicroscopic photograph of Group IV CH-normal saline following dye penetration (Group IV sample no. 1: 0.3 mm).

based intracanal medicaments were placed showed apical leakage irrespective of the vehicle used. Result of the present study revealed that the type of vehicle used to obtain the CH paste influenced the sealing ability of the epoxy resin based root canal sealer.

In the present study Group III [Table/Fig-6] where no medicament was placed showed the least apical leakage amongst all experimental groups. This difference in leakage values may be attributed to presence of remnants of CH on the root canal walls when placed as an intracanal medicament; thus, preventing the sealer from penetrating into the dentinal tubules. The addition of CH increases the film thickness of AH Plus which may lead to a thicker layer of sealer placed on the root canal walls and increase the chances of voids being incorporated. On the contrary, Porkaew P et al., reported an improvement in the sealing quality of root canal fillings with different sealers when CH was used as a temporary dressing [15]. They suggested that the improved seal with plugs of CH was due to the fact that the plug provides a stop or matrix against which the guttapercha and sealer may be condensed more effectively. It was also reported that apical leakage was more in non-medicated control teeth than in those that received CH dressings [16].

In the current study amongst the three CH medicaments, CH-CHX when used as an intracanal medicament showed a significantly higher microleakage mean value of 2.47 mm as compared to the other groups with $p<0.001$. Higher concentration (2%) of CHX solution was mixed with CH in the present study. The probable reasons for increased leakage in CH-CHX group could be two fold. First being, sodium hypochloride when mixed with CHX leads to Parachloroanaline (pCA) generation [13]. Studies have reported that CHX when used at high concentrations (2% CHX) [5] may lead to prolonged pCA generation, causing both CHX and its by-products to remain in the root canal for a considerable period of time [13]. The pCA precipitate might be the probable cause affecting the sealing ability of the obturation by occluding the dentinal tubules and preventing the penetration of the resin based sealer. This precipitate was successfully dissolved in 0.1 mol/l acetic acid, but the discoloration persisted [17].

Secondly, CHX is known to have the property of substantivity and sustained release that could last as long as 12 weeks. The adsorption of CHX onto the canal walls may interfere with the sealing. Also, it is likely that alkalinizing the pH by adding CH to CHX leads to precipitation of the CHX molecules and thereby, decreases its effectiveness and causing interference in the sealing of the obturated material [1]. Similar results were obtained by Hamidi MR and Mahmoudi E et al., in his study in which the presence of intracanal CH-CHX medicament increased apical leakage when AH26, an epoxy resin based sealer was used [18]. However, in contrast to this study Wuerch RM et al., observed that 2% CHX gel when used as an intracanal medication for two weeks did not adversely affect the apical seal when obturated with AH Plus sealer and gutta percha [19]. Kontakiotis EG et al., also found that the paste made of CH-2% CHX gel can be proposed for use in clinical practice without affecting the sealing ability of root canal obturation [20].

Among the medicated groups; Vitapex, a combination of CH and oil based vehicle showed the least leakage. This can be attributed to the fact that non aqueous oil based vehicles have larger contact angles and hence, exhibit decreased wetting, implying that they do not wet the dentinal walls as efficiently as hydrophilic aqueous vehicles [21]. Based on this it can be hypothesized that oil based vehicles may not intimately contact the root canal walls due to the large contact angles and hence, can be removed more easily and efficiently from the canal walls as compared to the hydrophilic vehicles without compromising the obturation seal. However, further studies are required to justify the better removal of Vitapex from the canal walls as compared to the other groups.

India ink was used for the dye penetration test since the size of its molecules are less than or equal to 3 µm, and this ink can penetrate a 0.22 µm bacteria filter. Therefore, bacterial invasion of an apical

seal seems to be unlikely if this dye did not succeed in penetrating the root canal via a gap between the root canal filling and the canal wall [22]. Furthermore, India ink is not decolourized by CH [23], it does not stain the dentine and shows the leakage pattern only. In addition, the extent of dye penetration is easy to detect [23] even in the dentinal tubules [24].

The microleakage technique that was used in this study was a passive dye penetration and clearing technique. Although clearing technique is more accurate than transverse sectioning [25] as it allows the leakage to be visualized in one tenths of a millimeter, it does possess certain drawbacks like deficient demineralization leading to opaque areas compromising the final transparency of the specimen, difference in demineralization time depending on the mineral content of the tooth sample and the inability to quantify the volume of tracer ingress [26,27].

The retrieval of CH from the root canal prior to obturation bears major clinical implications on long term prognosis of the treatment. Also, consideration should be given to CH remaining in the root canal when selecting a root canal sealer. Further studies are recommended in order to develop agents or techniques to completely remove CH from the root canal walls. Moreover, the interactions of CHX with CH need to be further evaluated and correlated with the long term clinical outcomes.

CONCLUSION

Despite the limitations of this in vitro dye penetration study, it can be concluded that apical leakage was observed in all groups with or without placement of intracanal medicament. However, it was observed that in the absence of a medicament, the apical leakage was the least. The CH-CHX medicated group showed the highest leakage values which was statistically significant compared to the CH-saline and Vitapex groups.

Although leakage studies are controversial and should be interpreted with caution, under the conditions of this study it can be safely deduced that it is not only the CH powder but the vehicle used may also influence the apical sealing ability of guttapercha-AH Plus obturated canals.

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