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Evaluating the effect of calcium hydroxide based intracanal medicaments on the sealing ability of MTA based sealer – An *in vitro* study

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Abstract
Introduction: Calcium Hydroxide (CH) along with various other vehicles can be used as an intra canal medicaments.
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 MTA sealer – gutta-percha obturation
Materials and Methods: Crowns of 60 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 MTA 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 MTA sealer and guttapercha using lateral compaction technique. The specimens were immersed in rhodamine b 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 Turkey 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 – MTA sealer obturated canals.

Keywords: MTA sealer, Chlorhexidine, Saline, Vitapex

1. 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 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, propyleneglycol 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 suchas 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 possibleapical 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.

Hence, while placing an intracanal medicament it is important to consider its effect on leakage of the root canal system [3, 4].
The purpose of this study was to evaluate the effect of three CH based intracanal medicaments on the apical sealing ability of Fillapex–gutta-percha 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 fillapex gutta-percha obturated root canals.

Materials and methods

This *in vitro* study was carried out in the Department of Conservative Dentistry and Endodontics, Divya Jyothi College of Dental sciences and research, Modinagar, UP India. 60 permanent single rooted, non-carious human teeth with intactapices 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: They were resected 15 mm from the apex using a diamond disc with water coolant and was standardized 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: Biomechanical preparation was done using protaper next rotary files. 1 ml of 17% EDTA (Dent wash PDP, India) followed by 5 ml of 5% sodium hypochlorite, both agitated using passive ultrasonic irrigation (P5 Booster Suprasson, Satelec/Acteon, India) were used for removing smear layer. 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=10).

Control Groups

Group I (Positive Control): After 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 fillapex (Dentsply, Germany)-guttapercha using lateral compaction technique and the access cavities were sealed with Cavit G.
After 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 at37^{0}cand 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 Fillapex- gutta-percha 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 (2gm) 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 2 mm layer of temporary filling material, Cavit G. After that the roots were stored at 37 ^{0}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 Fillapex sealer and guttapercha cones using lateral compaction technique and sealed with 2 mm of Cavit-G. Teeth were stored for 72 hours at 37 ^{0}C and100% 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 nailvarnish except at the apical 2 mm.

Dye Penetration Test: The teeth were immersed in rhodamine b and placed in the incubator at 37 ^{0}C for one week [5]. 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. Following dehydration, the teeth were transferred to methyl salicylate (Amrut Industrial Products, India) for two hours to allow diphanization or until the teeth were cleared. The teeth were dried and analyzed under 10X stereomicroscope to assess the extent of dye penetration in each group expressed in millimeters [6].

Statistical analysis

Statistical analysis was carried out by one–way ANOVA followed by Post-hoc Tukey test. The level of significance was set at $p< 0.05$.

Table 1:

Group	N	Mean	SD
Group I: Positive control	10	4.01	1.06
Group II: Negative control group	10	0.6	0.10
Group III: Without CH based intracanal medicament	10	0.68	0.28
Group IV: CH in normal saline	10	1.01	0.58
Group V: CH in 2% CHX gel	10	2.53	1.37
Group VI: Vitapex	10	0.59	0.75
Total	10	1.38	1.40

Result: Group 1 specimens ie positive control group showed complete leakage through out the length of the root canal (4.01±1.06mm) whereas the group 2, negative control group showed minimal leakage (0.6±0.10mm).

Among the medicated group, group vi medicated with vitapex showed the least leakage (0.59±0.75mm) and group v CH-2% CHX showed highest leakage (2.53±1.37mm).In group III mean peneteration of dye (0.68±0.28mm) and in group IV, it is(1.01±0.58mm).

Discussion

For successful root canal treatment, through removal of intra canal medicaments from the dentinal walls is required to ensure a three Dimensional seal. In studies it was shown that residual medicaments might compromise the adaptation of filling material and hence the seal of obturated root canal [7]. This causes the ingress of microorganisim or tissue fluids into canal space and leads to periapical inflammation.

MTA Fillapex is newly introduced sealer which has good physical and biological properties, hence used for the study. L imited studies are available about the sealing and dentinal tubule penetration ability of MTA Fillapex. Camilleri *et al*. evaluated the sealing ability of MTA Fillapex and Pulp Canal sealer using fluid filtration technique and reported that the sealing ability of both sealers was comparable [8]. Kuçi *et al*. evaluated MTA Fillapex sealer's penetration ability and stated that MTA Fillapex, compared to AH26, was associated with greater sealer penetration when used with cold lateral compaction technique [9]. The teeth were stored in an incubator at 37 ^{0}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 has proven to be one of the most effective combinations in removing CH based intracanal medicaments [9, 10]. 17% EDTA in conjunction with passive ultrasonic irrigation has shown promising results on debris and smear layer removal [11].

Studies have reported that CH could take upto 10 days to disinfect dentinal tubules infected by facultative bacteria as reported by few authors. Hence, in the current study CH was placed for a period of 14 days [12, 13].

In our study, it was observed that all experimental groups in which CH 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 MTA fillapex 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 [14, 15].

In the present study amongst the three CH medicaments, CH-CHX when used as an intracanal medicament showed a significantly higher microleakage mean value of 2.53 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 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 [16, 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. 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, 19, 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.

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 [21]. Further studies are recommended in order to develop agents or techniques to completely remove CH from the root canal walls.

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.

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